MINDD Conference
Sydney May 2009

INFECTIONS, ANTIBIOTICS, DYSBIOSIS & MINDDD:

Feeding our Bugs

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Infections

- Last 30 years: dramatic increases in illnesses and infections in children
- Otitis media increased from an uncommon illness (1970s) to now affecting 90% children under the age of 2 years
- Increases in hospital admissions for respiratory infections and gastroenteritis, 4.5%/year

'Big Four'

Same time frame: significant increases in incidence of

- Asthma
- Allergies: now up to 50% children, with 20% children with food allergies
- ADHD – to 10%, learning difficulties, 20%, speech delay 5%
- Autism, to 1/100

Infections and Neurodevelopmental disorders

- Association between infections in childhood and speech difficulties in boys
- Espec associations with ear infections (OM), 'mental health disorders, asthma, allergies
- Recurrent OM infancy also correlated with lower IQ scores, poor school performance, behavioural difficulties
- High correlation between prevalence OM and autism
- Correlation with high levels of antibiotic use and autism

Infections and the Brain

4 main mechanisms by which infections can affect brain function

1. Direct infection –eg viral encephalitis, bacterial meningitis
2. Toxin-mediated effects
3. Immune-mediated effects – eg autoantibody formation against brain cells
4. Disruption of normal metabolism and nutrition

Infections in Autism

Overt Infections

- Overt infection common in history of children prior to diagnosis of autism
- most common - otitis media (AOM)
- recent study 206 children under age of 3 with autism :
- average of nearly 10 bouts of AOM, received an average of 12 courses of antibiotics
- over 1/3 all given to children under 12 months

Otitis Media

- previously incidence of respiratory tract infections and otitis media peaked 4 to 6 years old
- now peak incidence between 6 and 12 months of age.
- Otitis media now said to occur in 48% children within first 6 months
- in 79% within 1st 12 months
- in 91% by age 24 months, .
- compares to 1989 figures: 62% of children by 12 months, 83% at age 3 years

Microbiology of AOM

- Bacterial - 75% (aspiration of middle ear fluid)
- *Strep pneumoniae* the major bacterial infecting agent
- Nasopharyngeal carriage of *Strep pneumoniae* extremely common, especially in young children.
- Nasal carriage identified as the first step in the acquisition of pneumococci and a necessary first step for invasive pneumococcal disease.

Strep Carriage

- Most children become nasal carriers of *Strep pneumoniae* at least once during 5 month period.
- Acute sinusitis in previous 3 months the major associated factor with carriage of *Strep pneumoniae*
- Otitis media in the past 3 months the only associated factor with carriage of antibiotic resistant *Strep pneumoniae.*
- Connection between nasal strep, otitis media and sinusitis

Biofilms

- Bacteria live in biofilms
- Sessile colonies surrounded by mucus slime
- Protected from predation by protozoa, antibiotics, antibodies, immune cells
- Antibiotic resistant – factor 1000x
- Cause 80% culture-negative, antibiotic resistant, chronic infections

Gastric Reflux and Infections

- Gastro-oesophageal reflux increasing in incidence in infants in recent years
- Now a major cause of morbidity and failure to thrive, particularly in neurologically impaired children.
- Has been linked with asthma, respiratory infections, childhood chronic sinusitis and AOM
- Reflux part of clinical spectrum of cow's milk allergy and intolerance

Infections, Reflux, Gastric Acid Inhibitors and Autism

- (2006 study) - correlation with use of GAH in infancy for GORD and increased rates of gastroenteritis (2x, to 50%) or pneumonia (5x, to 12%)
- " increased risk might result from overgrowth of intestinal pathogens (in the stomach) in a low-acid environment",
- intestinal pathogens might colonise the oral space and subsequently be aspirated
- anecdotally- rates of reflux, diagnosed GORD and use of PPI's high in infancy in children who later develop autism

Antibiotics

- Recurrence rate otitis media post antibiotics 2-6x cf placebo
- Antibiotic treatment for otitis media increases nasopharyngeal colonisation of non-pneumococcal alpha-haemolytic streptococcus at 2 months
- Antibiotic resistance in these strains is becoming very common
- Implications for respiratory infections not clear but increasing incidence respiratory infections and gastroenteritis (4%/year)

Antibiotics and Allergy

- Several studies linking prenatal and early infancy antibiotics to later allergy and asthma
- Meta-analysis of 8 studies
- receiving at least 1 antibiotic before 12 months old doubled risk of childhood asthma
- risk dose dependant
- each extra course during first year of life increases risk 1.16 times

Antibiotics and Allergy

- Maternal antibiotics in utero induces Th2 response in infant (immune shift)
- dose-related response
- increased risk asthma, hayfever, eczema, respiratory tract infections, candida

Antibiotics and the Immune System

- Antibiotics promote TH2 immune shift
- Change in gastrointestinal flora thought to be major reason behind TH2 immune system shift documented post anti-biotics.
- Antibiotics can induce increased intestinal permeability

Developing Picture:

- Early infancy – frequent breastfeeding difficulties, early formula
- Reflux, colic, treated with PPI
- Recurrent infections, biofilms
- Frequent antibiotic use, change in gastrointestinal flora, further Th2 shift
- Failure maturation of Th1 response, Th2 immune skewing
- Gut-based immune based dysfunction
- Loss of oral tolerance
- Food allergies, sensitivities

Immune System in Autism

Gut-Based Immune Responses in Autism

- Levels of all TH2 cytokines significantly ↑: (IL-4,5,13)
- ↑activation both TH1, TH2; TH2 predominance, no compensatory ↑ regulatory IL-10
- Th2 shift - ↓CD4+ T cells, ↑IL-4, TNF, ↓IFN-gamma,↓IgA,↑IgE (Gupta S)
- ↑antibody-producing B cells 20%, ↑ NK cells 40%
- ↑ total serum proteins
- ↑ albumin, gammaglobulins, partic IgG, IgG2, IgG4

Gut Immune Responses in Autism

- Recent study 100 autistic children on unrestricted diets, 77 on restricted diets, (controls with and without food sensitivities or restricted diets):
- challenge with bacterial toxins or milk proteins resulted in strong pro-inflammatory response, less able to down-regulate
- "may indicate the intrinsic natures of dyregulated innate immune responses in autism spectrum disorder children (with gastrointestinal involvement)

Autoantibodies in Autism

- High levels of autoantibodies against myelin basic protein (MBP) and neuron axonal filamentary protein in children with autism (60 to 70%) correlate with raised measles, HHV-6 antibody titres
- various other antibodies to neuronal tissue also documented
- recent study of 171 autistic individuals found 'high levels of antibodies against brain tissue', one particular unidentified protein apparently involved
- Antibodies also to frontal cortex ,5HT1A receptors, cerebellar neurofilament

Immunology in Autism: autoantibodies (cont)

Antibodies to 3 cross reactive peptides also raised in children with autism cf controls (IgG, IgA, IgM)

- chlamydia pneumoniae (CPP)
- streptococcal M protein (STM6P)
- milk butyrophilin (BTN)

- *Streptococcal spp* and *enterococcal spp* have been found to be elevated in large scale studies assessing common pathogens in cow's milk

The Gut in Autism

"All diseases begin in the gut"
Hippocrates 460-370 BC

Gut Dysfunction in Autism

Gut abnormalities documented:

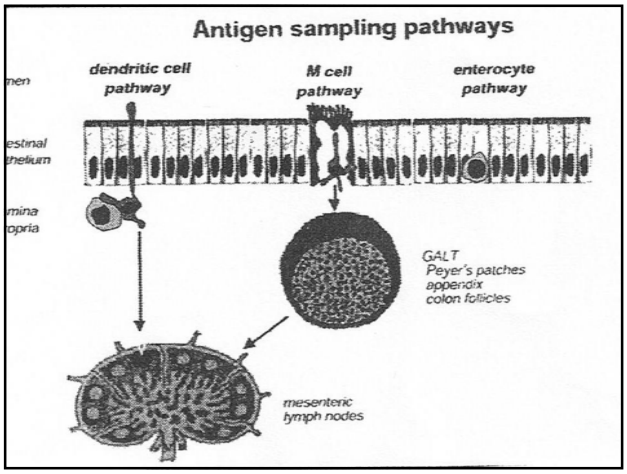
- gastric hypochlorhydria with resultant raised pH (Horvath)
- duodenitis (Horvath)
- reduced intestinal disaccharidase enzyme function (Horvath) & dipeptidase function
- colitis, lymphoid hyperplasia (Wakefield,Krigsman)
- increased intestinal permeability (Eufemia)

Infancy, Immunity and Gut Microflora

- Children born with Th2 dominance
- Th1 responses mature in first year or so of life
- mainly in response to microbial antigens
- dependant on growth of symbiotic gut bacteria
- reduction symbiotic bacteria (lactobacilli) in Caesarian-born infants

Gut Microflora and Gut Immunity

- Recent studies indicate that antigen-presenting cells (APC) - dendritic cells, M cells - routinely sample intestinal microflora
- APC release appropriate cytokine stimulus, dependant on strain of bacteria
- induce either inflammatory (IL-2,4,5,6,13) or anti-inflammatory (IL-10, TGF-beta) response



GALT

- Oral tolerance - mechanism by which immunological tolerance to food antigens and commensal gut flora is induced
- typically, absence of appropriate oral tolerance results in exaggerated Th2 responses

Intestinal Flora

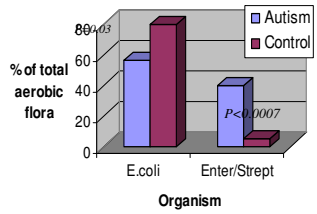
- Intestinal flora affects gut permeability
- failure of the mucosal immune system usually begins with various changes to the population of microflora & subsequent dysfunction of the mucosal epithelial barriers
- in the absence of beneficial intestinal microflora, disturbance in intestinal absorption of macromolecules is more severe than in its presence

Intestinal Flora

- Healthy adult gut - av 1.5-2kg bacteria, 10-50% total body cells, over 600 species
- symbiotics, indigenous - *Bifidobacteria, Lactobacilli, Propionobacteria,* physiological strains *Ecoli, Peptostreptococci, Enterococci*
- opportunistic - *Bacteroides, Peptococci, Staphylococci, Streptococci, Bacilli, Clostridia,* Yeasts *(esp Candida), Enterobacter (Proteus, Citrobacter Klebsiella),* etc
- transitional - us gram-negative bacilli

Alterations of the faecal aerobic microbial flora in patients with autism (n=36)

Butt HL, Cosford RE, Roberts TK, Dunstan HR,McGregor NR, Ellis L
University of Newcastle Bioscreen



- The mean % distribution of *Escherichia coli* for the autistic patients was 56.3% of the total aerobic flora, compare to 80% in healthy control subjects (p=0.03).

Enterococcus/Streptococcus spp., was significantly higher in counts (40.1%) in the autistic patients than in healthy subjects (5%). (p<0.0007)

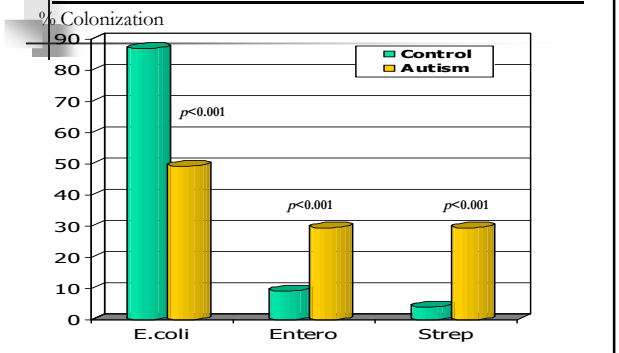
Autism

Retrospective, multi-center (NSW, VIC, & QLD), comparative study of the faecal microbial flora of Autistic Patients and control subjects in, 2004-2005

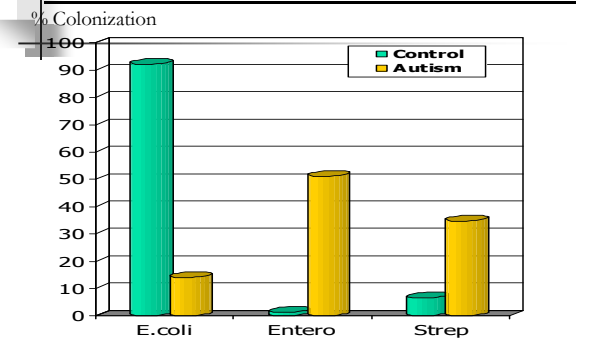
Butt H.L.[1] Emms T.M.[1], Cosford, R.[2], Duff, J.[3], & Patterson D[4].

Bio21, Molecular Science & Biotechnology Institute, & *Bioscreen*, University of Melbourne [1];
Northern Beaches Care Centre, Mona Vale, NSW[2];
Behavioural Neurotherapy Clinic, Doncaster, VIC[3];
Secrenase Medical Center, Runaway Bay, QLD[4].

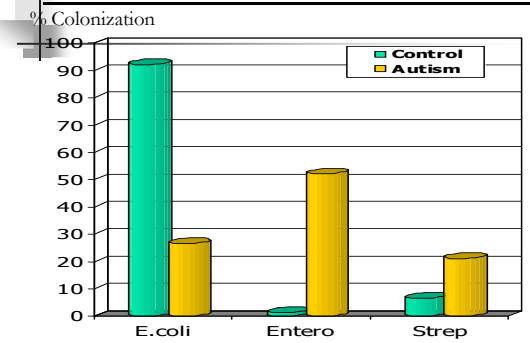
Colonization pattern of faecal aerobes:
NSW autistic patients (n=27) and control subjects (n=117)



Colonization pattern of faecal aerobes:
QLD autistic patients (n=45) and control subjects (n=117)



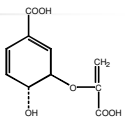
Colonization pattern of faecal aerobes:
VIC autistic patients (n=16) and control subjects (n=117)



- Predominant Streptococcal overgrowth seen was non-pneumoccal alpha-haemolytic or non-haemolytic streptococcus
- Similar to pattern of overgrowth seen in nasal carriage post antibiotic therapy
- ?Swallowed down to gut in biofilm

The importance of *E.coli* metabolites in human metabolism

- *E.coli* and other Gram negative bowel organisms produce chorismate



Chorismate is the precursor for :

- 4-aminobenzoate —·—·→ *folic acid*
- 4-OH-benzoate —·—·→ *ubiquinol (CoQ_{10})*
- anthranilate —·—·→ *tryptophan*
- prephenate —·—·→ *tyrosine, phenyalanine*
- isochorismate —·—·→ *menaquinone (Vit K)*

Functions of Symbiotic Flora

Antibacterial action:

- bifidobacteria lactis -ability stimulate innate & Th1 activity, enhance production antimicrobial cytokines (IL-1, IL-2, IFN-gamma, TNF-alpha), helps restore Th1 deficiency
- eg normal human flora capable of permanently eradicating *Clostridia difficile* from gut
- Lactobacillus (GG) also been demonstrated to reduce Clostridia in gut

Gastrointestinal Dysfunction in Autism

- Inflammation, increased gastrointestinal permeability
- reduction in enzyme function
- gastrointestinal dysbiosis with colonosis
- predominant overgrowth of streptococcal/enterococcal species, loss of E coli, lactobacilli sp
- disruption of normal gastrointestinal flora function & metabolites

Gut-Brain Axis

- "gut-brain interactions may be central to abnormal neural development and the subsequent expression of aberrant behaviors"

Andrew Wakefield

Gut- Brain Axis

4 mechanisms

1. Enteric nervous system, neurotransmitters
2. Gut- Associated-Lymphoid Tissue, gut-based immune responses
3. Increased intestinal permeability, increased direct toxin and antigen passage
4. Disruption usual gut bacterial metabolism and nutrients

Metabolic Abnormalities

- Numerous metabolic abnormalities demonstrated in children with autism
- "often manifest complex biochemical, metabolic and immunologic abnormalities that a primary genetic cause cannot readily account for"

Enterococcus/Streptococcus and D-Lactic Acid

- Facultative anaerobes
- Homofermentative- produce only lactic acid from glucose fermentation
- (NMR exo-metabolic profiling) – significantly more lactic acid than *Ecoli*
- Predominantly D-lactic acid
- Associated with decreased faecal pH

D-Lactic Acidosis

- Short Bowel Syndrome (SBS) – adult patients – headaches, weakness, cognitive impairment, fatigue, pain, severe lethargy related to D-lactic acidosis)
- Increased intestinal permeability reported in these cases believed to be due to increased colonisation of lactic acid producing Gram-positive bacteria Increased permeability would likely result in increased absorption of microbial metabolites including D-lactic acid
- D-lactate poorly metabolised in humans as lack enzyme D-lactate dehydrogenase

Organic Acidosis in Autism

- 1/3 low bicarb
- 2/3 raised anion
- Consistent with chronic metabolic organic acidosis,
- Streptococcal/enterococcal overgrowth usual in cases organic acidosis
- (personal data)

Gastrointestinal Dysfunction in Autism

- Increased gastrointestinal permeability
- gastrointestinal dysbiosis with colonosis
- predominant overgrowth of streptococcal/enterococcal species
- markers for malabsorption
- disruption of normal gastrointestinal flora function and metabolites
- metabolic sequelae- organic acidosis

Streptococcus and Autoantibodies

Strep and Autoimmune Disease

Strep species known to be associated with neuro-psychiatric disorders, abnormal movements, auto-immune phenomenon

- scarlet fever
- post-streptococcal glomerulonephritis
- rheumatic fever
- Sydenham's chorea (associated with rheumatic fever)

PANDAS

- PANDAS- Paediatric Auto-Immune Neuro-psychiatric Disease Associated with Streptococcus
- OCD exacerbations follow GAS infections, assoc abnormal behaviours, emotional lability, separation anxiety, attentional difficulties
- associated psychiatric disturbances - OCD, generalised anxiety, depression, conduct disorders, hyperkinetic disorders

PANDAS- ADHD, Autism

- Raised strep titres (ASOT, antiDNAse B) correlate with ADHD`(excluding OCD, tics)
- raised ASOT correlated with increased basal ganglia volumes
- raised strep titres common in ASD (c. 50%), often when no recent history of overt streptococcal infection (personal data)

Streptokinase and DPPIV

- Streptococcal enzyme (streptokinase) binds to DPPIV enzyme, more strongly than gluten, casein
- DPPIV is proline endopeptidase – tissue enzyme which hydrolyses bonds containing proline: necessary for degradation of gluten and casein
- DPPIV also CD26 lymphocyte marker;
- Plays key role in growth & differentiation of lymphocytes; T cell mediated immune responses and cytokine production
- Potential for streptococcal overgrowth to interfere with degradation of gluten and casein and immune function
- Binding of SK to DPPIV results in autoantibody formation to peptides and tissue antigens
- 'dysfunctional membrane peptidases and autoantibody production may result in neuroimmune dysregulation and autoimmunity'

Streptococcus and Immune Responses

- Strongly immunogenic, antibodies frequently cross react with human tissue
- Generally, bacteria elicit B cell response, viruses T cell response
- All *streptococci* - ability to nonspecifically stimulate T cell as well as B cell response
- Inadequate T cell or B cell response could result in chronic *streptococcal* infection,
- Could then result in depletion both B cell and T cell immune mediators

Streptococcal toxins

- Known strep toxins - eg erythrogenic toxin of scarlet fever, b-haemolytic membrane toxin of b-haem strep
- streptokinase - ↑inflammatory mediators TNF alpha, IL1, IL6 (Tcells)
- neuraminidase - aid in establishment of viral infections
- NADase- depletes NAD, necessary for recycling glutathione
- glutathione peroxidase - necessary for virulence

Streptococcal Toxins & effects

- Streptococcal pyogenic toxins -'superantigens' - stimulate certain T cells to proliferate without processing the toxin
- ↑ TNF alpha reduces lymphocyte glutathione
- ↑ TNF alpha implicated in Tourette's, facial tics, ACD, schizophrenia

Chronic Streptococcal Effects

In gut-

- damage to intestinal wall - glycosaminoglycans
- stimulation of mucus
- increased intestinal permeability
- reduction in disacccharidase enzymes (glycosidases)
- XS antigenic stimulation of GALT
- reduction symbiotic bacteria
- reduction of bacterial metabolites
- bacterial toxins - cross gut wall
- XS D-lactate

Chronic Streptococcal Infection

- antibody production, cross-reactions human tissue (kidney, heart valves, ?brain)
- systemic inflammation (TNF-alpha, IL-1, IL-6)
- depletion of NAD (numerous enzyme systems including recycling glutathione)
- direct depletion of glutathione (via glutathione peroxidase)
- reduces resistance to viral infections (neuraminidase)

Streptococcus - colonisation

- *Strep pneum* normally colonises nasopharynx 20-40% children
- prepubertal girls, vulvovaginitis strep sp is common
- vulvovaginitis GABHS seen only in this age group (vulvovaginal candidiasis not found in prepubertal girls)
- GBHS vaginal swabs 1/3 pregnant women

A Theoretical Model for the Aetiology of Autism

- Recurrent infections, commonly streptococcal, treated with antibiotics, result in loss of protective gut flora and overgrowth of predominantly streptococal species in gut
- loss of beneficial bacteria disrupts normal gut function and production and digestion of nutrients

- overgrowth of streptococcal species inflammatory to gut wall,resulting in loss of sulphated GAGs in intestinal mucus and increased gastrointestinal permeability
- increased permeability results in passage of macromolecules, abnormal immune activation and food intolerances, peptiduria

- streptococci toxigenic, resulting in numerous metabolic blocks, membrane dysfunctions
- possibly neurotoxic, resulting in abnormal neurological manifestations of Autism, ADHD, CFS
- immune response to streptococci result in cross-reactions with neuronal tissue, with further intracerebral irritation and inflammation
- excitotoxicity (NMDA receptor, glutamate), free radical damage (reduced glutathione) final common pathway in brain for these effects

Chronic Streptococcal Infection and Autism

- Results in widespread metabolic disruption
- inflammation, free radical damage
- auto-immune reactions
- affect brain development and function

Management

Basis: initially: nourish gut, cells, brain
reduce inflammation, oxidation

- Organic whole food 'Primitive' Traditional alkaline diet
- 5 P's
- gut: probiotics, colostrum, glucosamine, glutamine etc
- organic whole food-based supplements (high inherent antioxidant capacity, immune support phytonutrients)
- EFA
- Nutrients specific to nervous system (eg Mg, Zn, B6, SAME, glycine, gingko etc)
- Nutrients to enhance detoxification (eg selenium, N-acetyl cysteine, silymarin, glycine,zeolites etc)
- homeopathy

FOODS AND STREP

- Strep feeds on milk
- Strep feeds on sugar
- Strep produces enzymes which can interfere with breakdown of milk and wheat
- Strep can cause inflammation in the small intestine which can disrupt enzyme production (disaccharidases) and affect breakdown of carbohydrates
- Probiotics and fermented foods compete with strep in gut
- High alkaline diet counter acidosis produced by strep strep

Further

- 'chelation' – nutrient based, homeopathy

Also supportive therapies

- Primitive reflex integration – homeopathy, kinesiology, chiropractic, movement therapies
- Auditory/visual pathway integration – sound therapies, biofeedback

Always treat the whole child not just the symptoms

Neuro-Immune Gastrointestinal Dysfunction Syndrome

- Theory: ADHD, Autism auto-immune and toxin-mediated disease processes in conjunction with malnutrition secondary to gastrointestinal dysfunction, in genetically susceptible individuals
- toxins - associated with streptococcal infections, streptococcal and food-derived toxins traversing intestinal barrier, other environmental toxins (mercury)
- streptococcal and milk antigens activate gut-associated lymphoid tissue (GALT) and cross-react with neuronal antigens

Summary

- Multisystem disorder: neurological
  immunological
  gastrointestinal
  biochemical

Metabolic Immunological Neurological Digestive Disorder

MINDD

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