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MEDICINE

IACC Workshop 2014:

Under-Recognized Co-Occurring Conditions in Autism Spectrum Disorder (ASD)

Immune system and Autism Spectrum Disorders

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IACC WORKSHOP 2014:

Immune Disorders

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Department of Internal Medicine and the M.I.N.D. Institute
University of California Davis

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Disclosures:

Conflict of Interest: None

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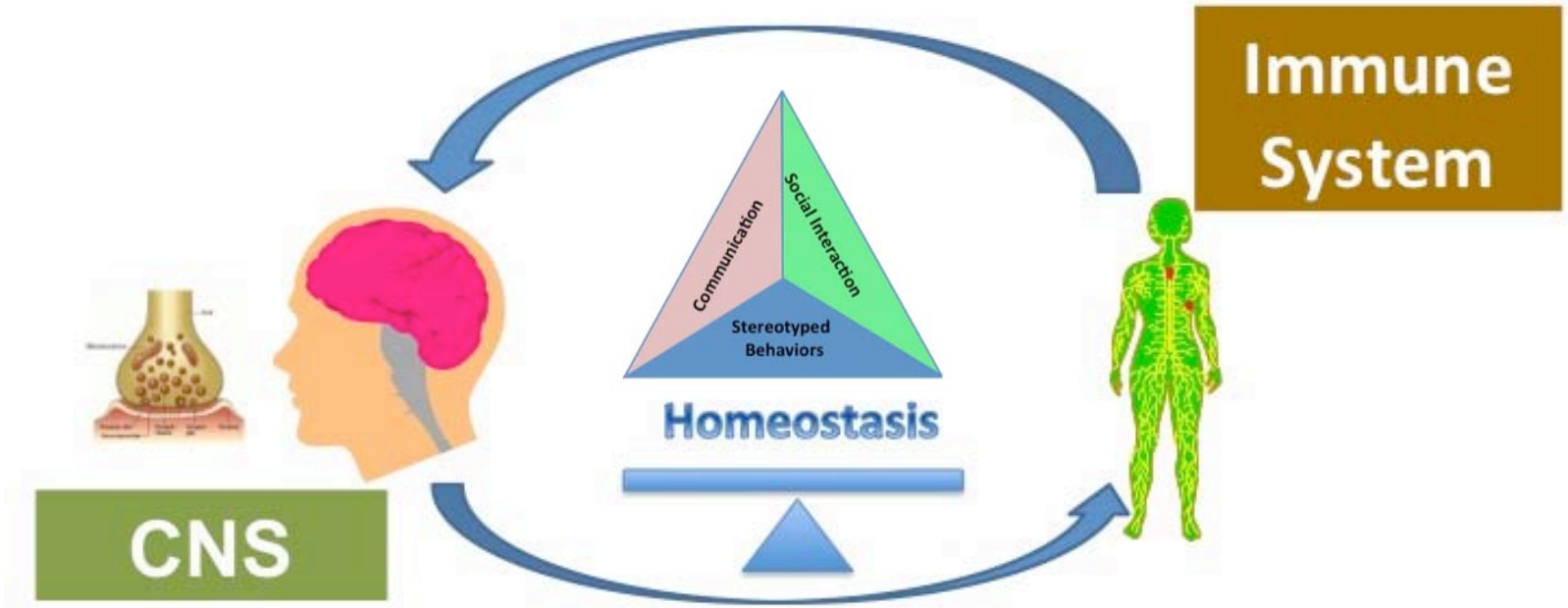
- *Peter Emch Fund for Autism Research*
- *Cure Autism Now/Autism Speaks*
- *National Institutes of Health, National Institutes of Mental Health (HHSN271200700179)*



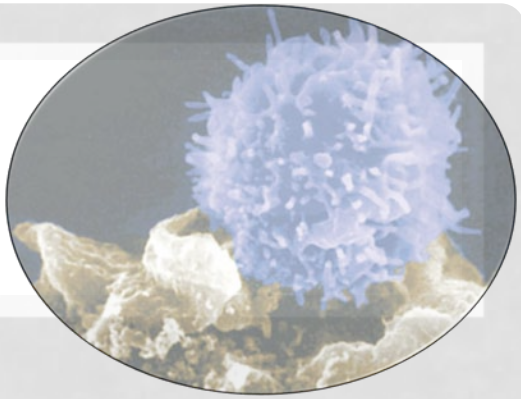
Gene-Environment Interaction:
Etiologic Model of Autism Spectrum Disorders

Genetic Factors

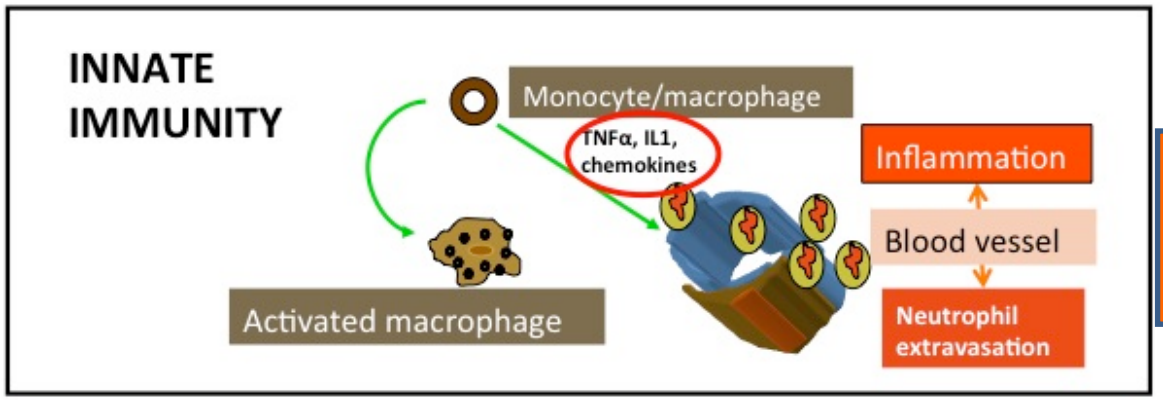
Immunity

Environment

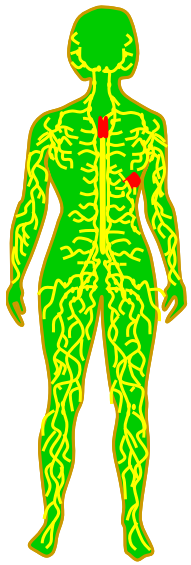
THE FIRST QUESTIONS:



- What is the level of evidence for immune system involvement in ASD?
- Is there evidence for a common biologic cause of the co-morbidity?
- What are the autism-specific features that affect proper diagnosis and treatment of the co-morbidity?

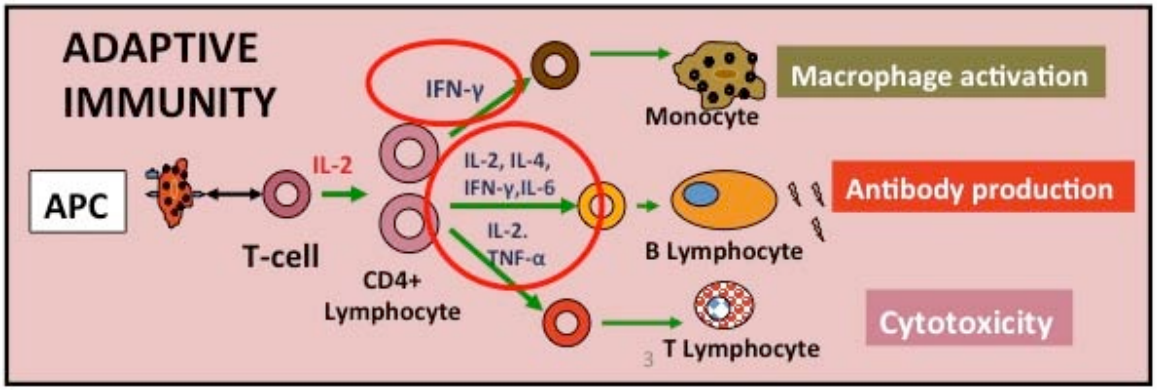


Non-specific Response



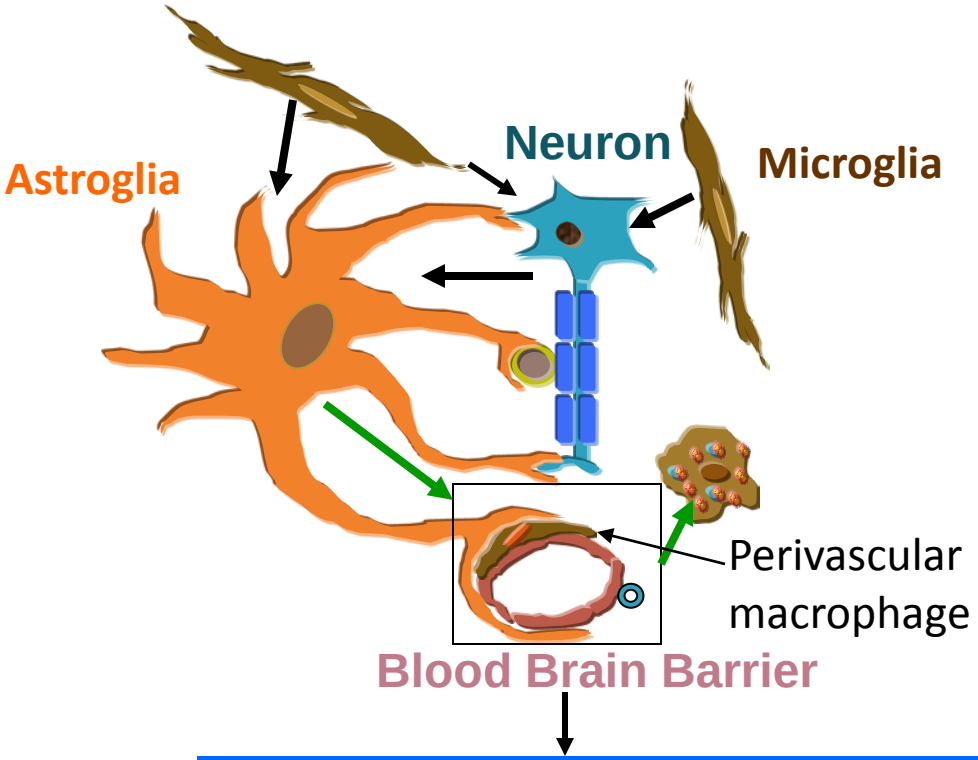
Homeostasis

Genetic factors
Trauma
Infections
Malignancy
Autoimmunity
Metabolic
others



Highly-specific Response

The Neuroimmune System

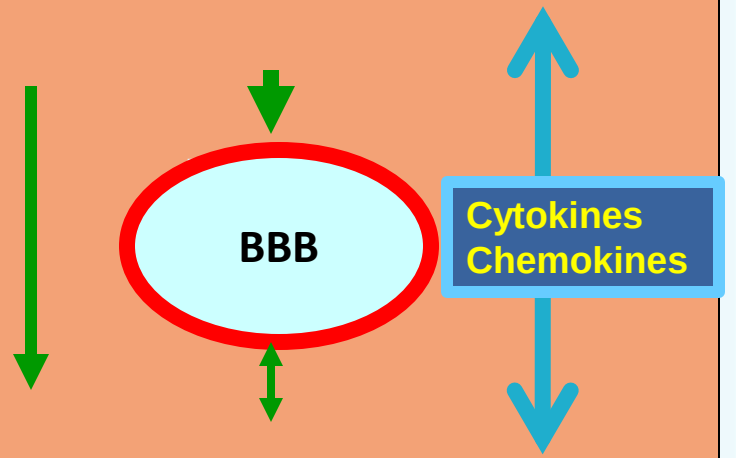


Modulation of immune responses
Lymphocyte/monocyte trafficking

CENTRAL NERVOUS SYSTEM

Innate immunity

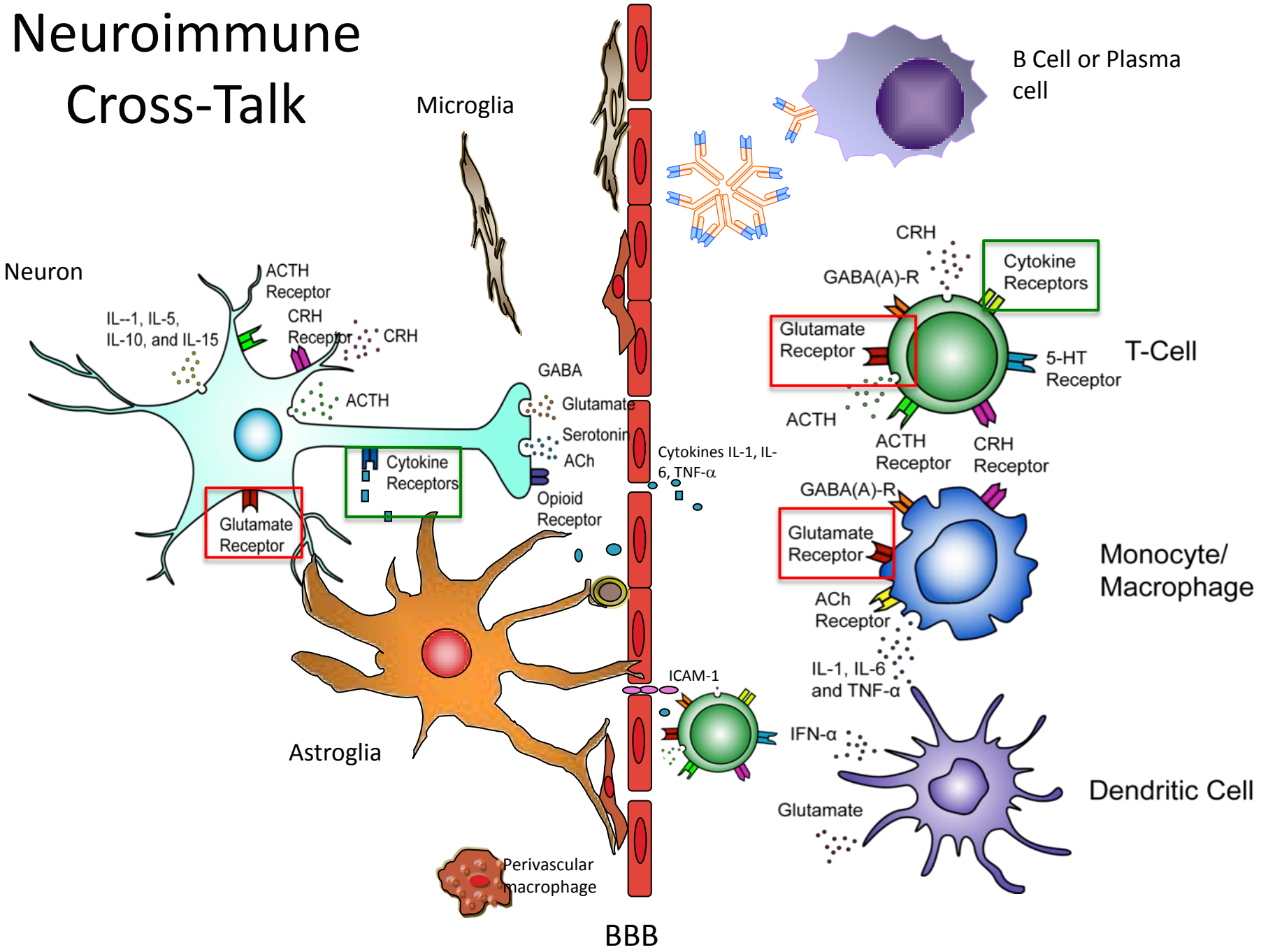
Microglia & astroglia



Adaptive immunity

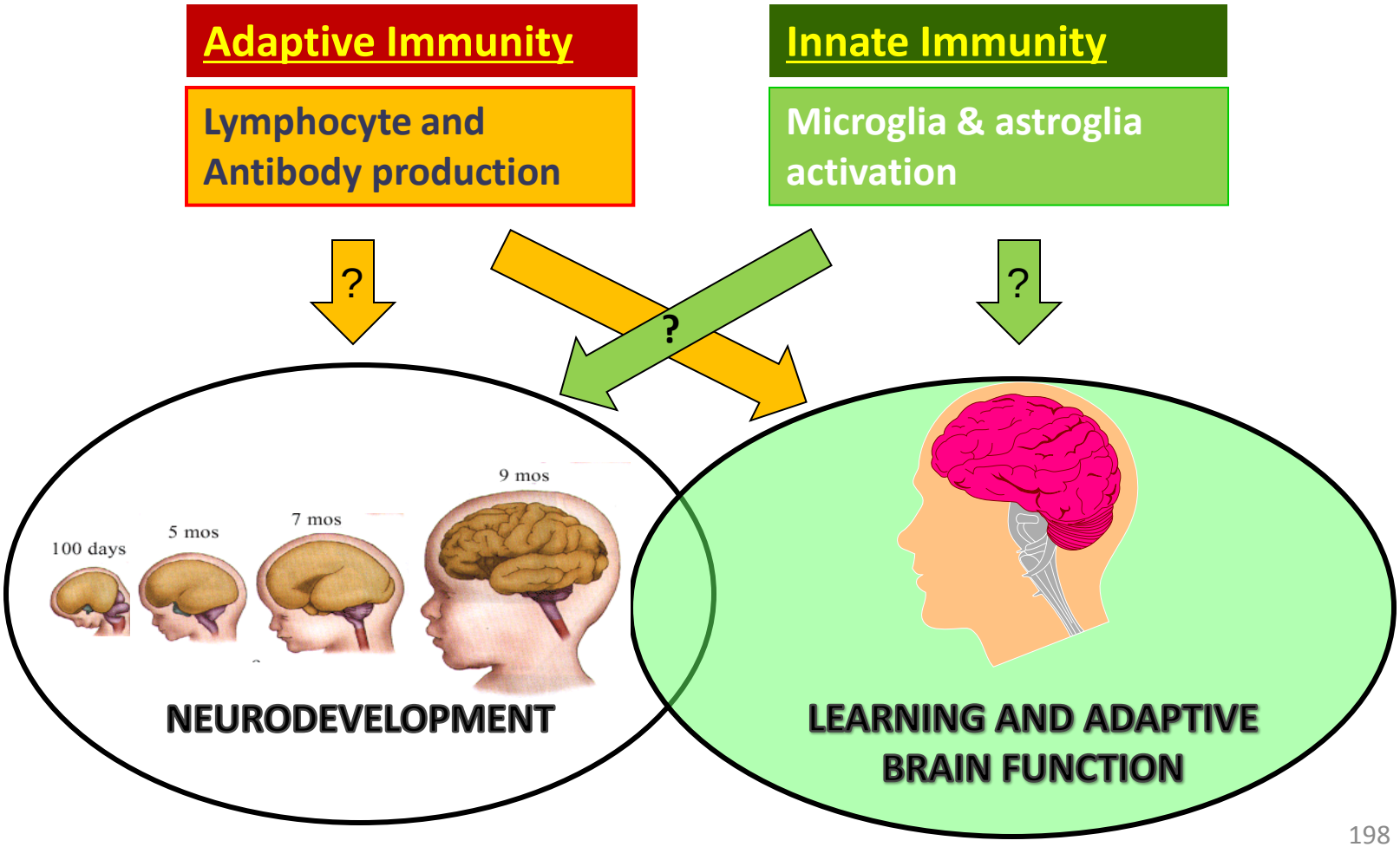
Specific T cell and antibody responses

Neuroimmune Cross-Talk

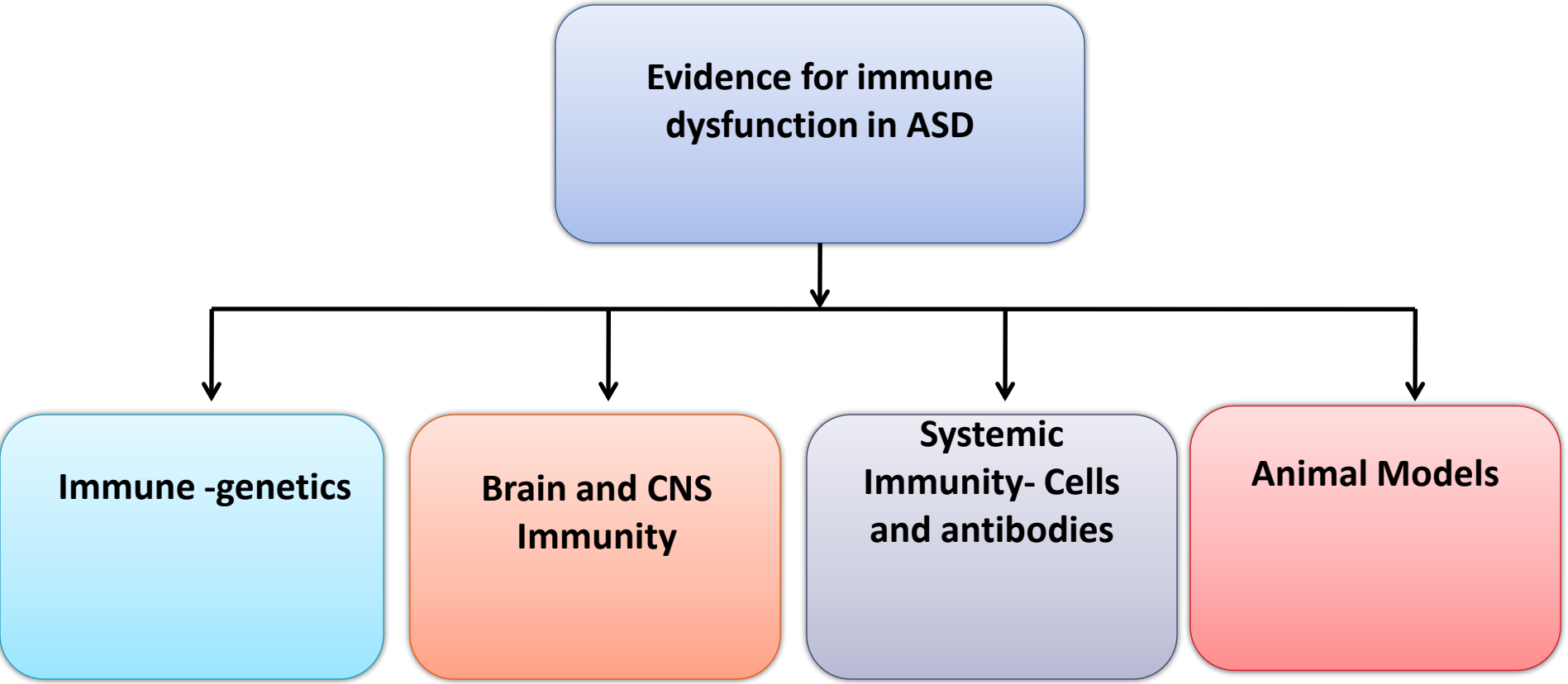


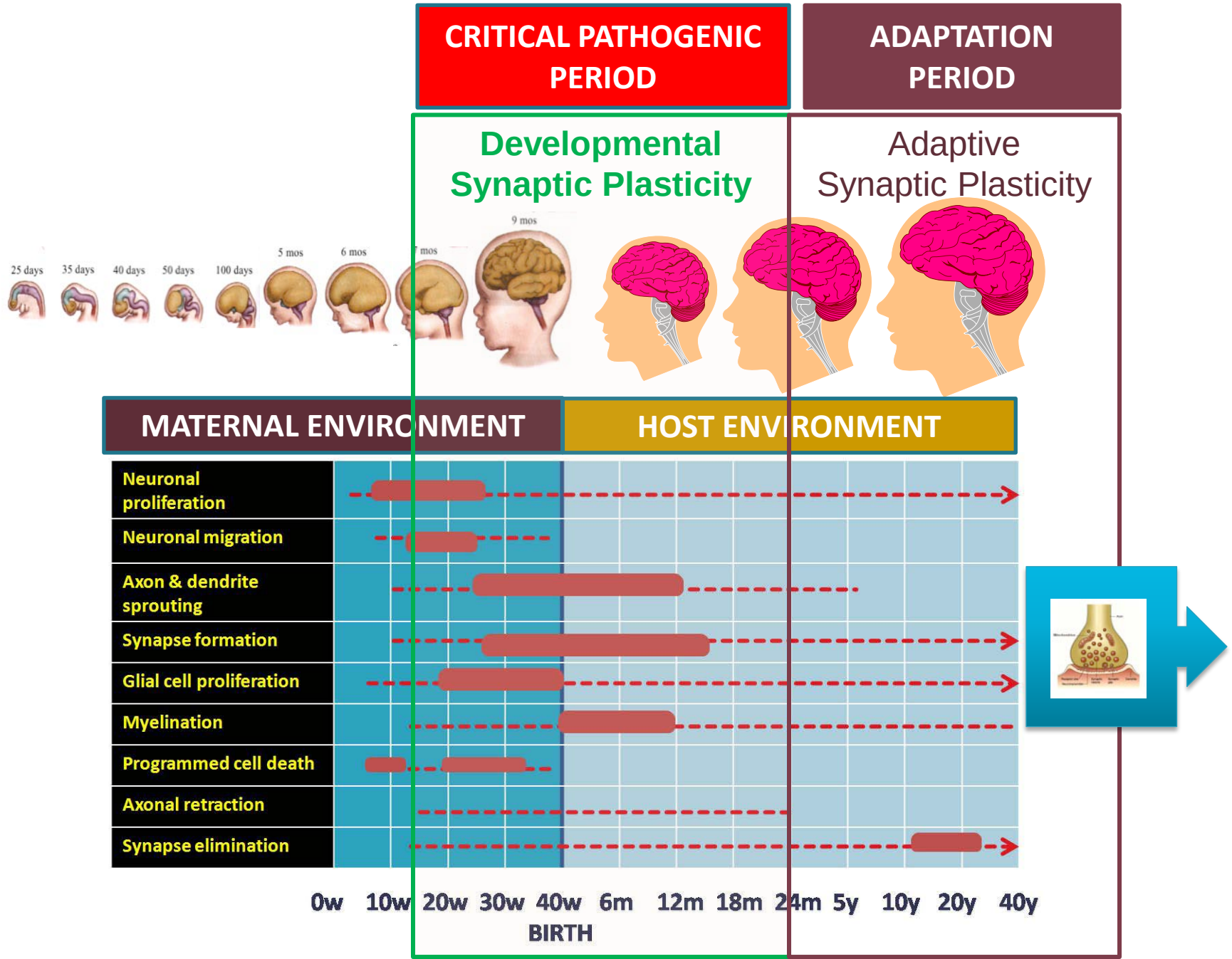
Key Questions:

Are immune or neuroimmune mechanisms involved in pathogenesis of ASD? What is the evidence?



Evidence for immune dysfunction in autism from many avenues




CRITICAL PATHOGENIC PERIOD
ADAPTATION PERIOD
Developmental Synaptic Plasticity
Adaptive Synaptic Plasticity
25 days
35 days
40 days
50 days
100 days
5 mos
6 mos
9 mos
MATERNAL ENVIRONMENT
HOST ENVIRONMENT
Neuronal proliferation
Neuronal migration
Axon & dendrite sprouting
Synapse formation
Glial cell proliferation
Myelination
Programmed cell death
Axonal retraction
Synapse elimination
0w
10w
20w
30w
40w
6m
12m
18m
24m
5y
10y
20y
40y
BIRTH

Question:

Are maternal immune mechanisms involved in pathogenesis of ASD?

Adaptive Immunity

Lymphocyte and Antibody production

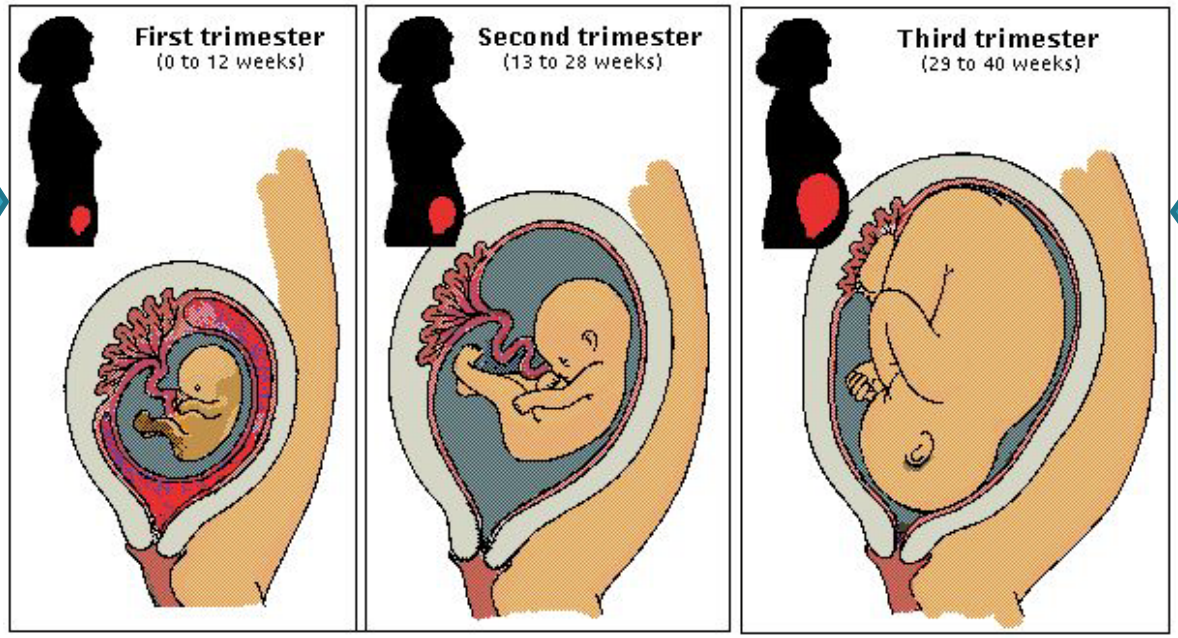
Innate Immunity

Microglia & astroglia activation

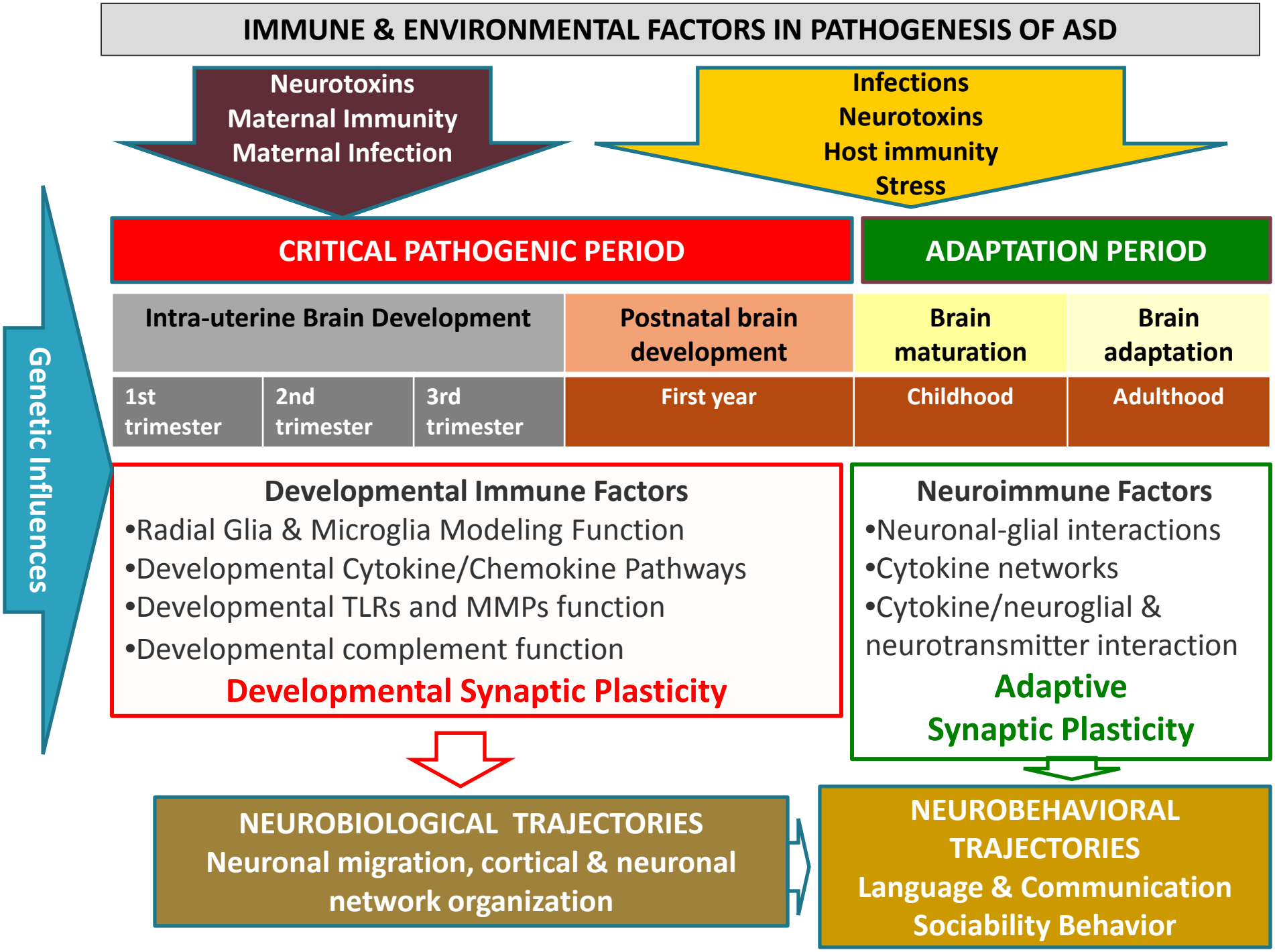
?

?

Maternal Infection



Auto-immunity


IMMUNE & ENVIRONMENTAL FACTORS IN PATHOGENESIS OF ASD
Neurotoxins
Maternal Immunity
Maternal Infection
Infections
Neurotoxins
Host immunity
Stress
Genetic Influences
CRITICAL PATHOGENIC PERIOD
ADAPTATION PERIOD
Intra-uterine Brain Development
Postnatal brain development
Brain maturation
Brain adaptation
1st trimester
2nd trimester
3rd trimester
First year
Childhood
Adulthood
Developmental Immune Factors
•Radial Glia & Microglia Modeling Function
•Developmental Cytokine/Chemokine Pathways
•Developmental TLRs and MMPs function
•Developmental complement function
Developmental Synaptic Plasticity
Neuroimmune Factors
•Neuronal-glial interactions
•Cytokine networks
•Cytokine/neuroglial & neurotransmitter interaction
Adaptive
Synaptic Plasticity
NEUROBIOLOGICAL TRAJECTORIES
Neuronal migration, cortical & neuronal network organization
NEUROBEHAVIORAL TRAJECTORIES
Language & Communication
Sociability Behavior

What are the known immune mediated factors in ASD pathogenesis?

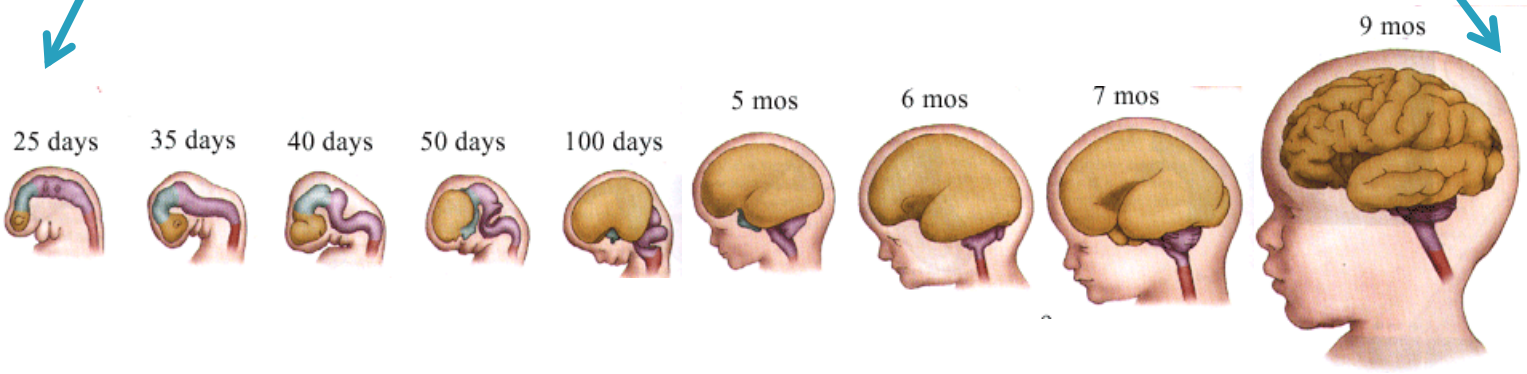
"Maternal autoimmunity in autism"

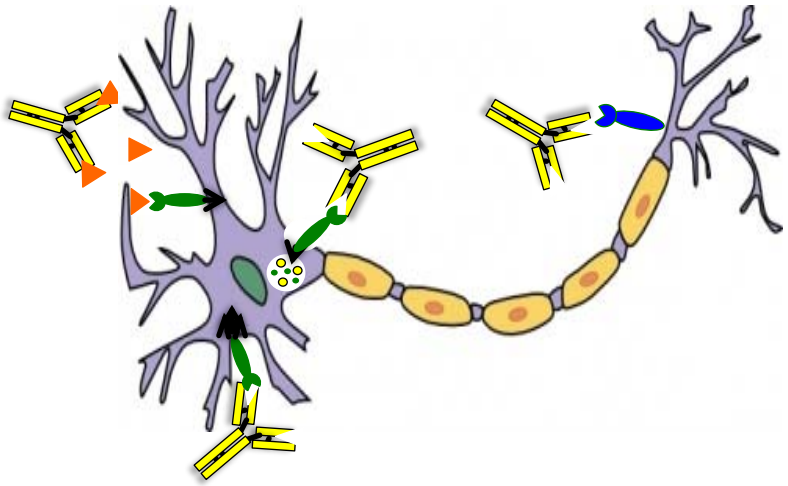
Judy Van de Water et. Al.
University of California at Davis,
Davis, California

Maternal Immunity

MATERNAL ENVIRONMENT | **ENVIRONMENT**

CRITICAL PATHOGENIC PERIOD





Maternal Autoantibody Related (MAR) Autism

Autoantibodies present in the circulation of mothers during pregnancy that recognize proteins in the developing fetal brain

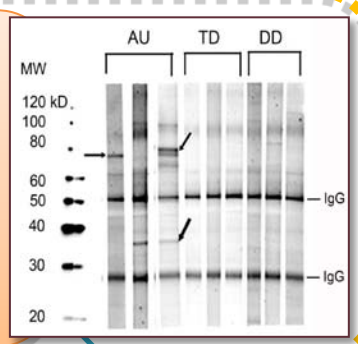
Maternal Anti-Brain Antibodies and ASD:

The studies behind the novel immune biomarker for autism risk

Epidemiology
Large population studies to identify potential risk factors for ASD

+

Initial finding: Immunology
Some mothers who have children with autism produce anti-brain antibodies

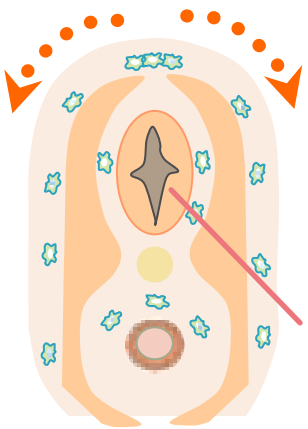


	Genetics	MRI	Basic Science	Animal Models	Brain Tissue Studies
Antibodies associated with regression and language deficits, stereotypic behavior	MET genetic variant associated with production of the anti-brain antibodies	Enlarged brain volume in male children prenatally exposed the antibodies	The antigenic targets of the antibodies have been identified	Animal models (2 monkey, 2 mouse) show behavioral changes after prenatal exposure to the antibodies; monkey model has also reported increased brain volume	Animal models provide tissue to explore brain pathology (ongoing)

Translational Potential:

- Identify kids with this sub phenotype and develop tailored behavioral treatment
- Screen women at risk and develop preventative strategies
- Define pathophysiology associated with these antibodies and develop therapeutic interventions

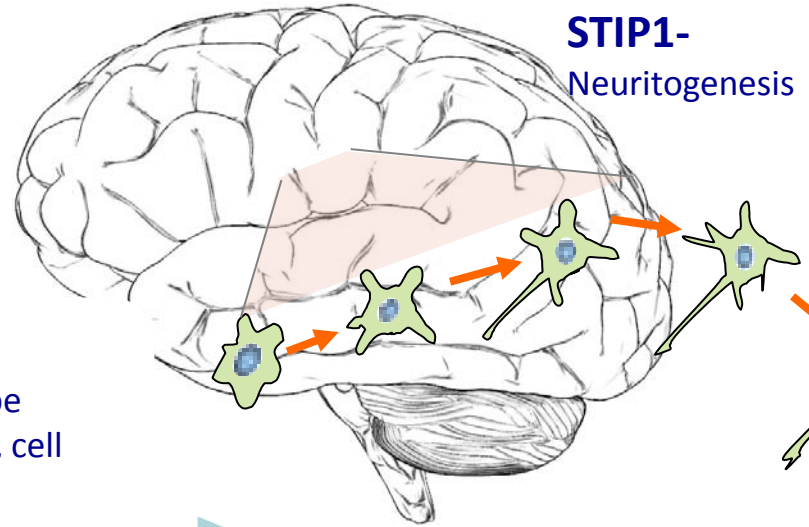
We Have Identified 7 Maternal Antibodies That Bind to Protein Targets Critical to Normal Brain Development*



Embryonic Cross-section

CRMP1-
Cell migration

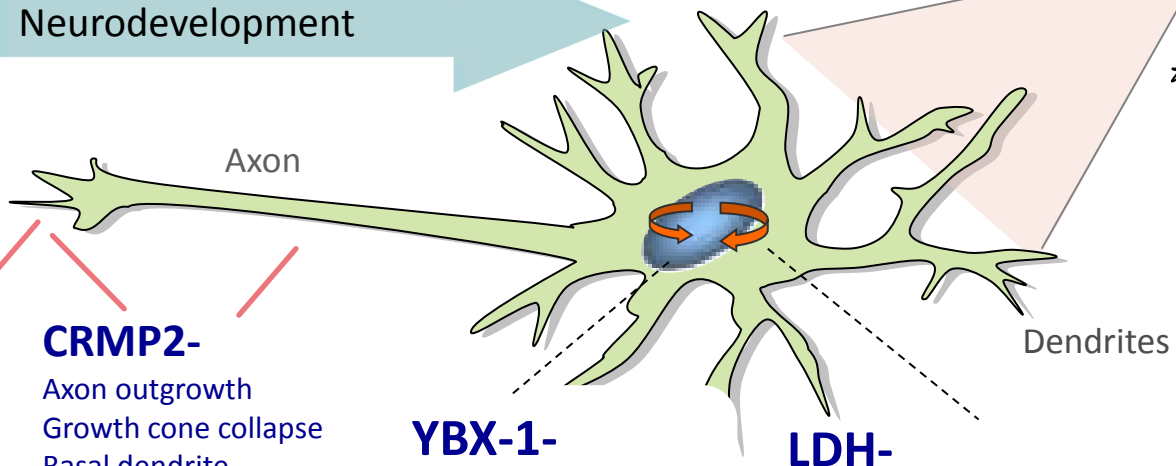
YBX-1-
Neural tube formation, cell division



STIP1-
Neuritogenesis

Cypin (GDA)-
Dendritic branching

Neurodevelopment



CRMP1-
Growth cone collapse

CRMP2-
Axon outgrowth
Growth cone collapse
Basal dendrite patterning

YBX-1-
Transcription regulation

LDH-
Metabolism

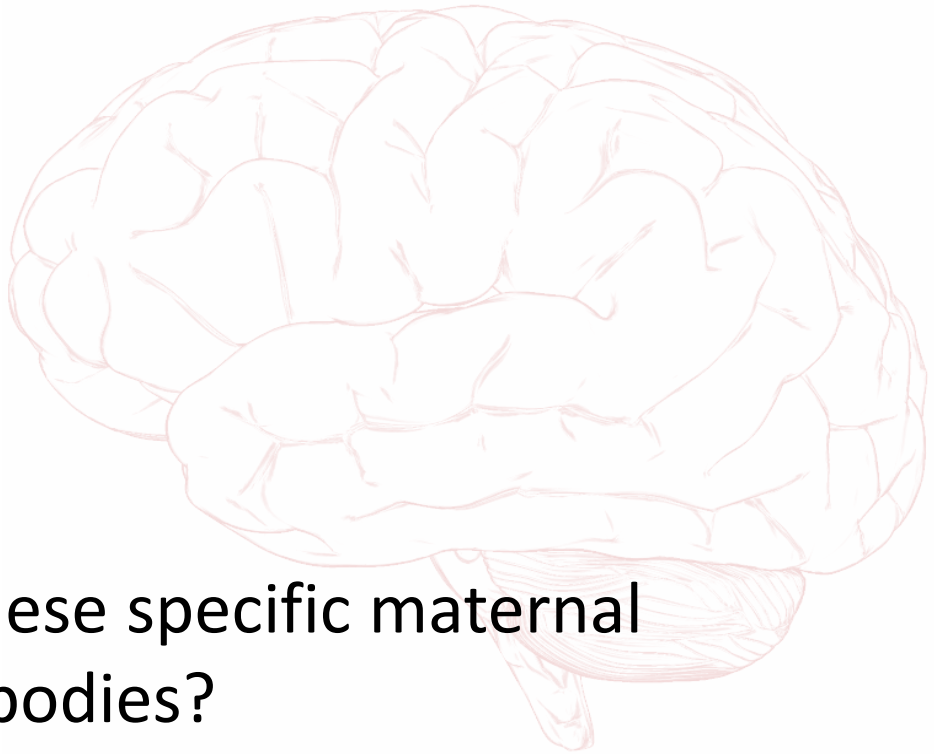
*Braunschweig, et al. ***Translational Psychiatry", July 9, 2013**

Specific MAR Antigens

# of Antigens	Antigen	% ASD (N = 241)	N	% TD (N = 147)	N	P Value
2	LDH + CRMP2	7%	17	0%	0	0.0004
2	STIP1 + CRMP2	10%	24	1%	1	0.0001
2	CRMP1 + CRMP2	7%	16	0%	0	0.0008
3	LDH + STIP1 + CRMP1	5%	12	0%	0	0.0045
3	Cypin + YBX1 + CRMP1	2%	5	0%	0	0.1615
3	Cypin + STIP1 + CRMP1	7%	18	1%	1	0.0025
3	Cypin + STIP1 + CRMP2	3%	8	0%	0	0.0267
3	YBX1 + STIP1 + CRMP2	3%	7	0%	0	0.0478
4	LDH + Cypin + YBX1 + STIP1	2%	5	0%	0	0.1615
4	LDH + Cypin + STIP1 + CRMP1	2%	5	0%	0	0.1615

Several Ab combinations are only found in mothers of ASD children

All specific combinations combined identify an association of MAR antibodies in ~23% of mothers with children on the ASD spectrum



What are the effects of these specific maternal autoantibodies?

-Do they have pathologic significance?
-do they affect how the brain develops?

Brain, Behavior, and Immunity xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Brain, Behavior, and Immunity

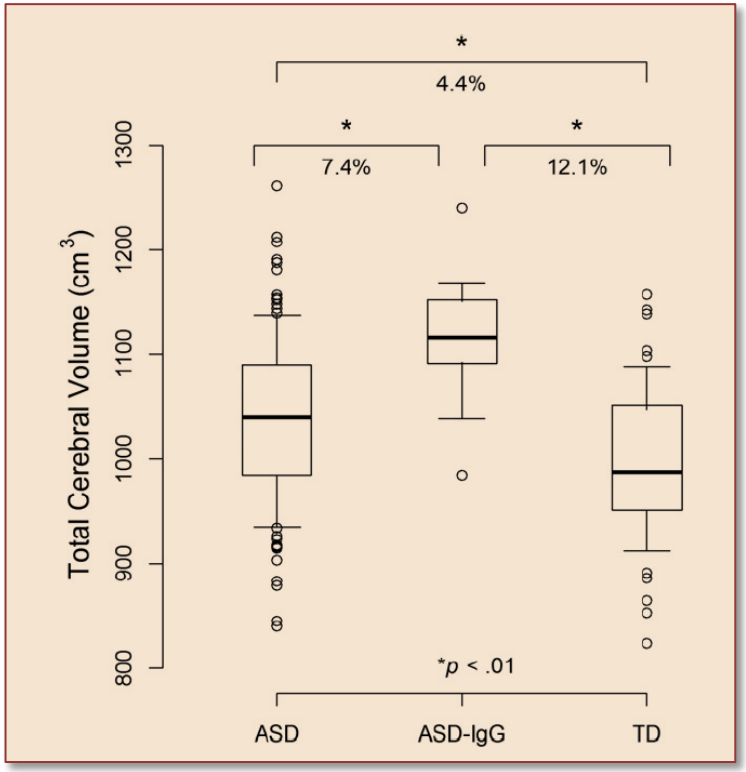
journal homepage: www.elsevier.com/locate/ybrbi

Short Communication

Maternal autoantibodies are associated with abnormal brain enlargement in a subgroup of children with autism spectrum disorder

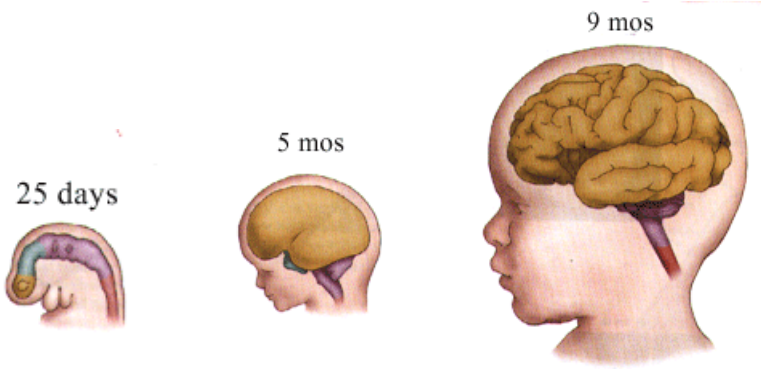
Christine Wu Nordahl [a,b,*], Daniel Braunschweig [c], Ana-Maria Iosif [d], Aaron Lee [b], Sally Rogers [a,b], Paul Ashwood [a,c], David G. Amaral [a,b], Judy Van de Water [a,c]

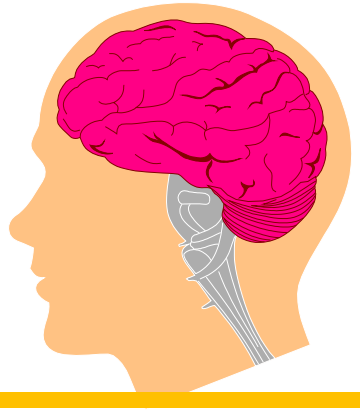
- Studied 181 2-4 YO male children (131 ASD, 50 typically developing (TD) controls) and evaluated total brain volume using structural magnetic resonance imaging (MRI).
- The ASD MAR group exhibited a **more extreme 12.1% abnormal brain enlargement** relative to TD controls.
- The remaining ASD children had a smaller 4.4% abnormal brain enlargement relative to TD controls.
- Lobar and tissue type analyses revealed that the frontal lobe is selectively enlarged
- **MAR autoantibodies may impact brain development leading to abnormal enlargement**.



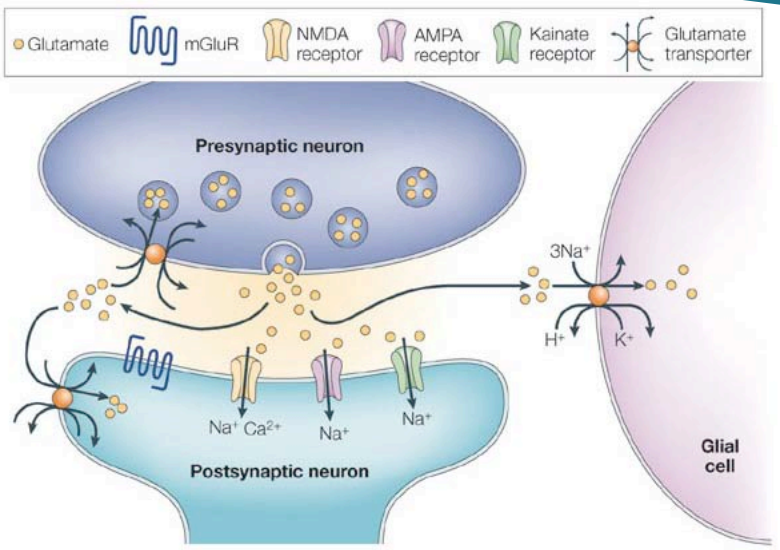
What is the pathology associated with these fetal brain specific auto-antibodies?

- Several passive transfer studies in mice and monkeys show evidence of MAR effects on behavior.
- Our first monkey model pilot study demonstrated behavioral changes in offspring following passive transfer of maternal IgG. Martin, L. A., et al 2008
- Our second monkey study by Bauman et al, Translational Psychiatry 2013, also demonstrated clear differences in social interaction.
- Maternal autism-associated IgG antibodies delay development, reduce social interaction, and produce anxiety in a mouse gestational transfer model (Braunschweig, et al. JNI, 2012).
- Increase in spontaneous grooming behaviors in response to a novel environment in mice exposed intraventricularly to MAR IgG. Comacho et al, Brain behav res. 2014.





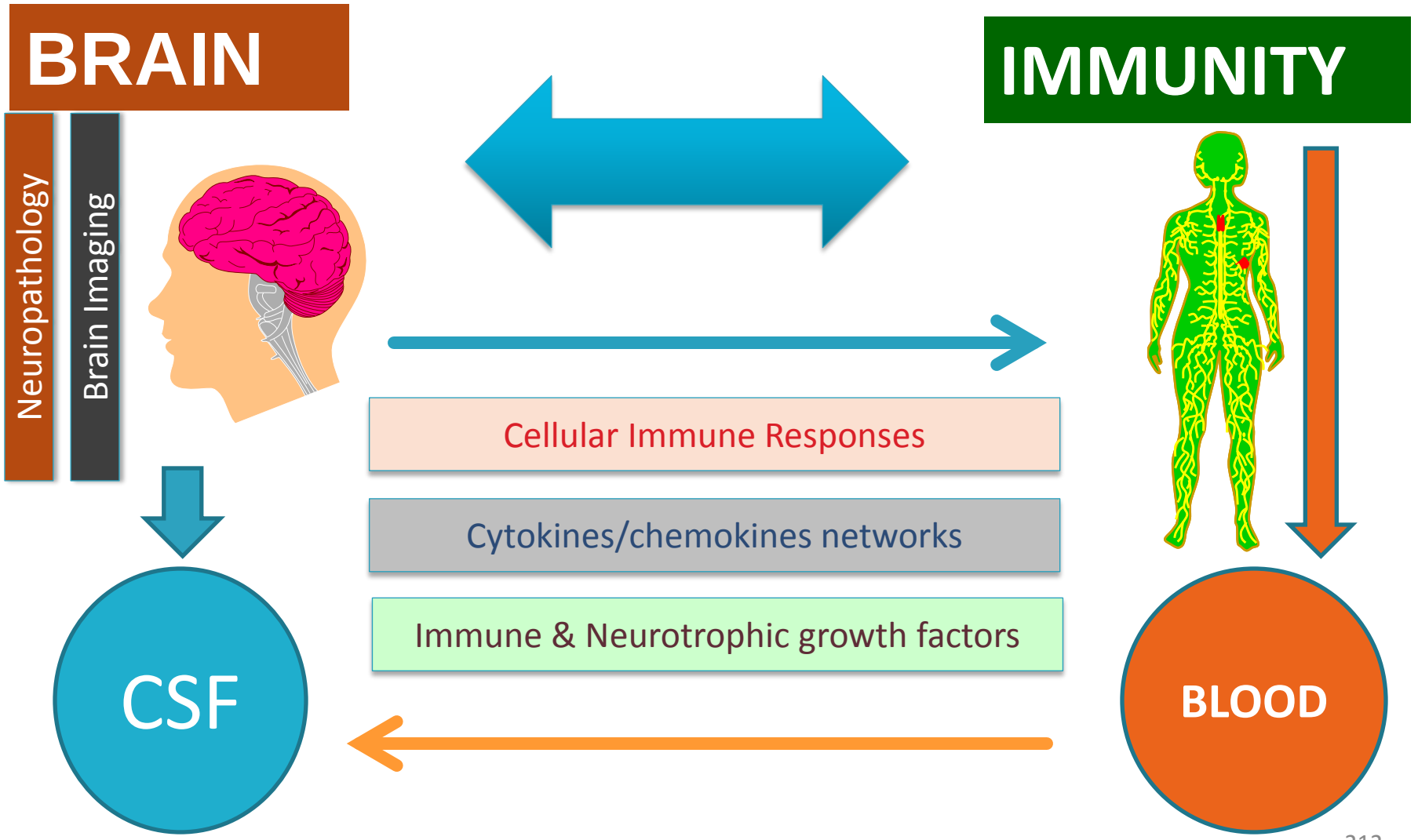
CRITICAL PATHOGENIC PERIOD				ADAPTATION PERIOD	
Intra-uterine Brain Development			Postnatal brain development	Brain maturation	Brain adaptation
1st trimester	2nd trimester	3rd trimester	First year	Childhood	Adulthood



Synaptic Plasticity
- Synaptic pruning
- Neurotransmitter homeostasis
- Synaptic stripping

Microglia
Astrocytes
Neuronal-neuroglia interaction
Cytokine/chemokine pathways

HOW TO STUDY NEURAL AND IMMUNE INTERACTIONS IN ASD?



Neuroglial Activation and Neuroinflammation in the Brain of Patients with Autism

Diana L. Vargas, MD,[1,2] Caterina Nascimbene, MD,[1–3] Chitra Krishnan, MHS,[1]
Andrew W. Zimmerman, MD,[1,4] and Carlos A. Pardo, MD[1,2,5]

Ann Neurol 2005;57:67–81

Microglial Activation and Increased Microglial Density Observed in the Dorsolateral Prefrontal Cortex in Autism

John T. Morgan, Gursharan Chana, Carlos A. Pardo, Cristian Achim, Katerina Semendeferi, Jody Buckwalter, Eric Courchesne, and Ian P. Everall

BIOL PSYCHIATRY 2010;68:368–376

Transcriptomic analysis of autistic brain reveals convergent molecular pathology

Irina Voineagu[1], Xinchen Wang[2], Patrick Johnston[3], Jennifer K. Lowe[1], Yuan Tian[1], Steve Horvath[4], Jonathan Mill[3], Rita M. Cantor[4], Benjamin J. Blencowe[2] & Daniel H. Geschwind[1,4]

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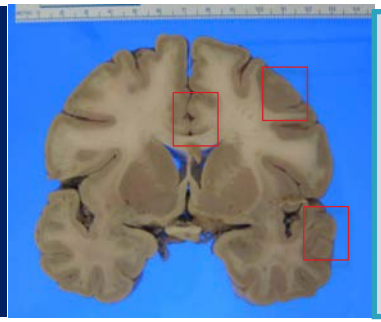
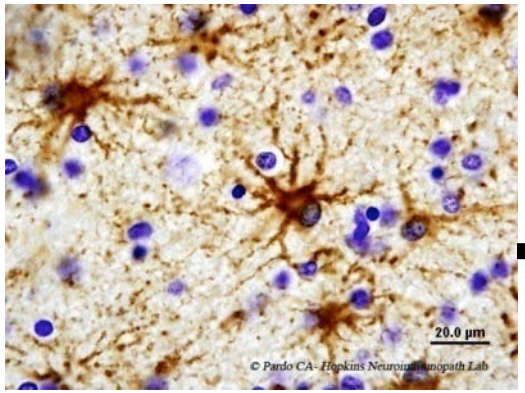
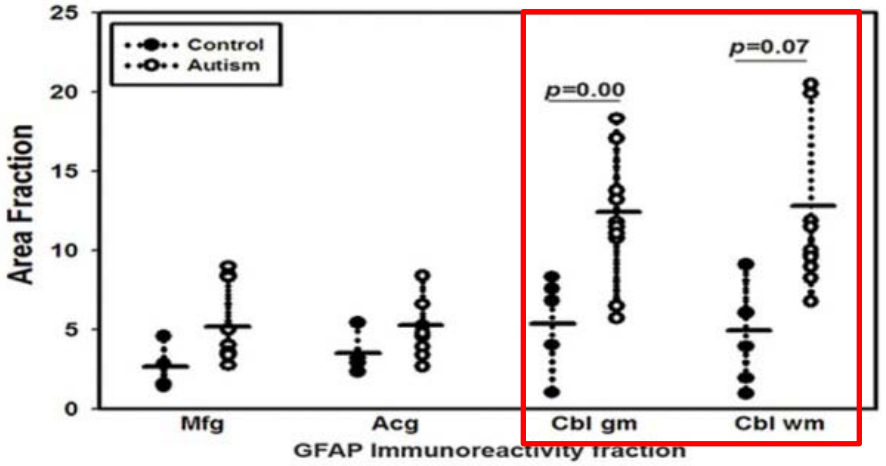
Assessment of Neuroglial reactions: Quantification of microglia and astroglia activation

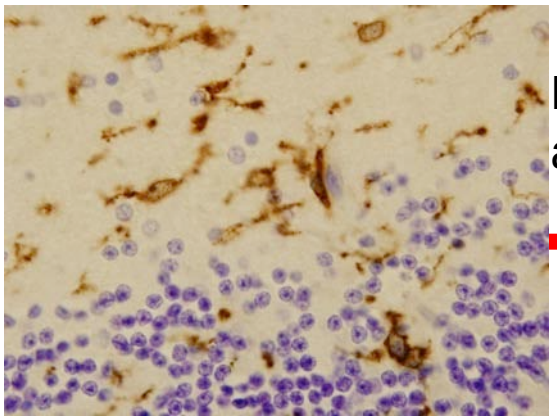


11 cases of autism
(age 5-44)
12 controls
Brain regions:
Frontal (MFG), cingulate (ACG), and cerebellum

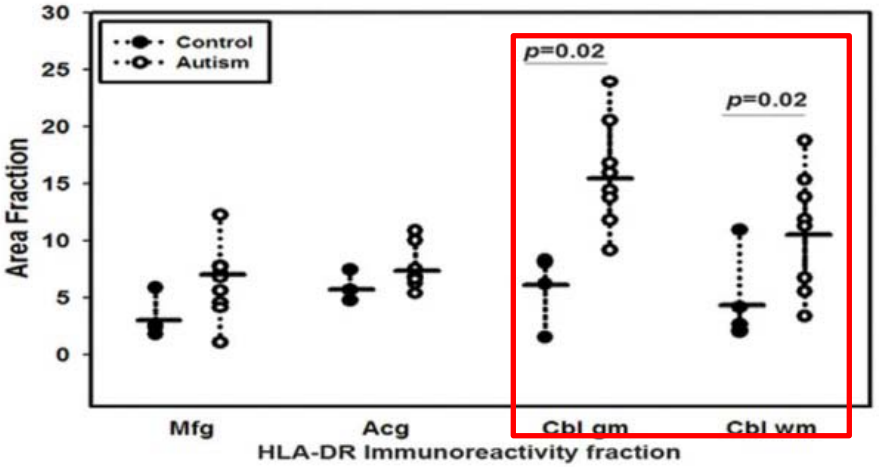


Astroglia activation



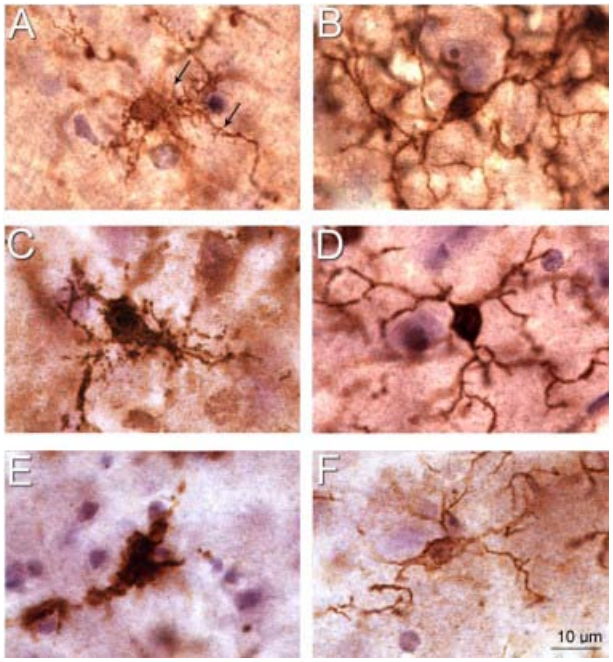


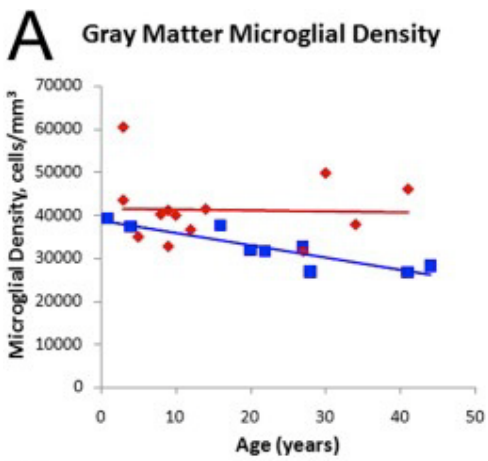
Microglia activation

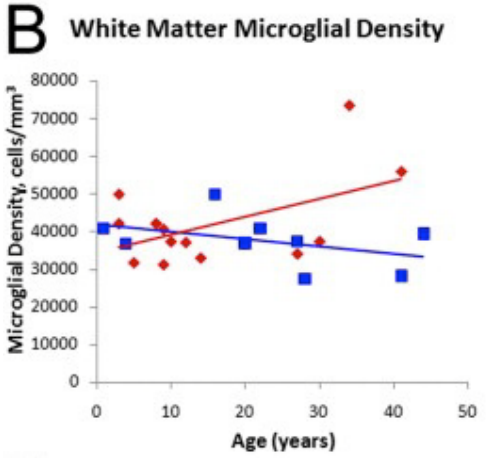


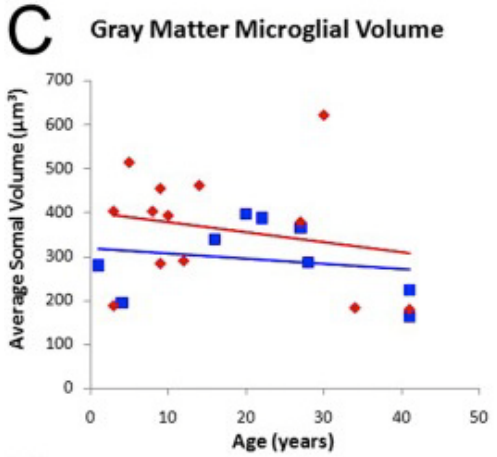
Vargas DL et al. Ann Neurol 2005

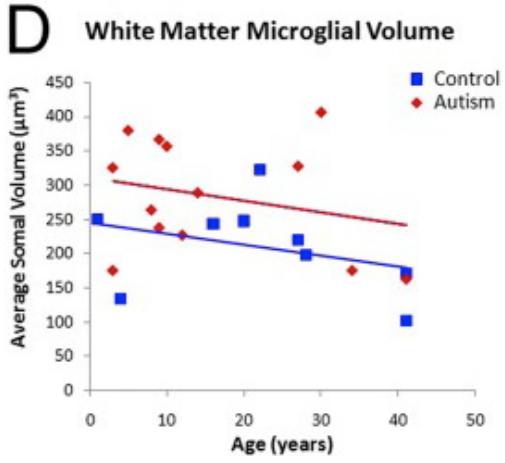
Microglia cell density increases in the cerebral cortex in ASD





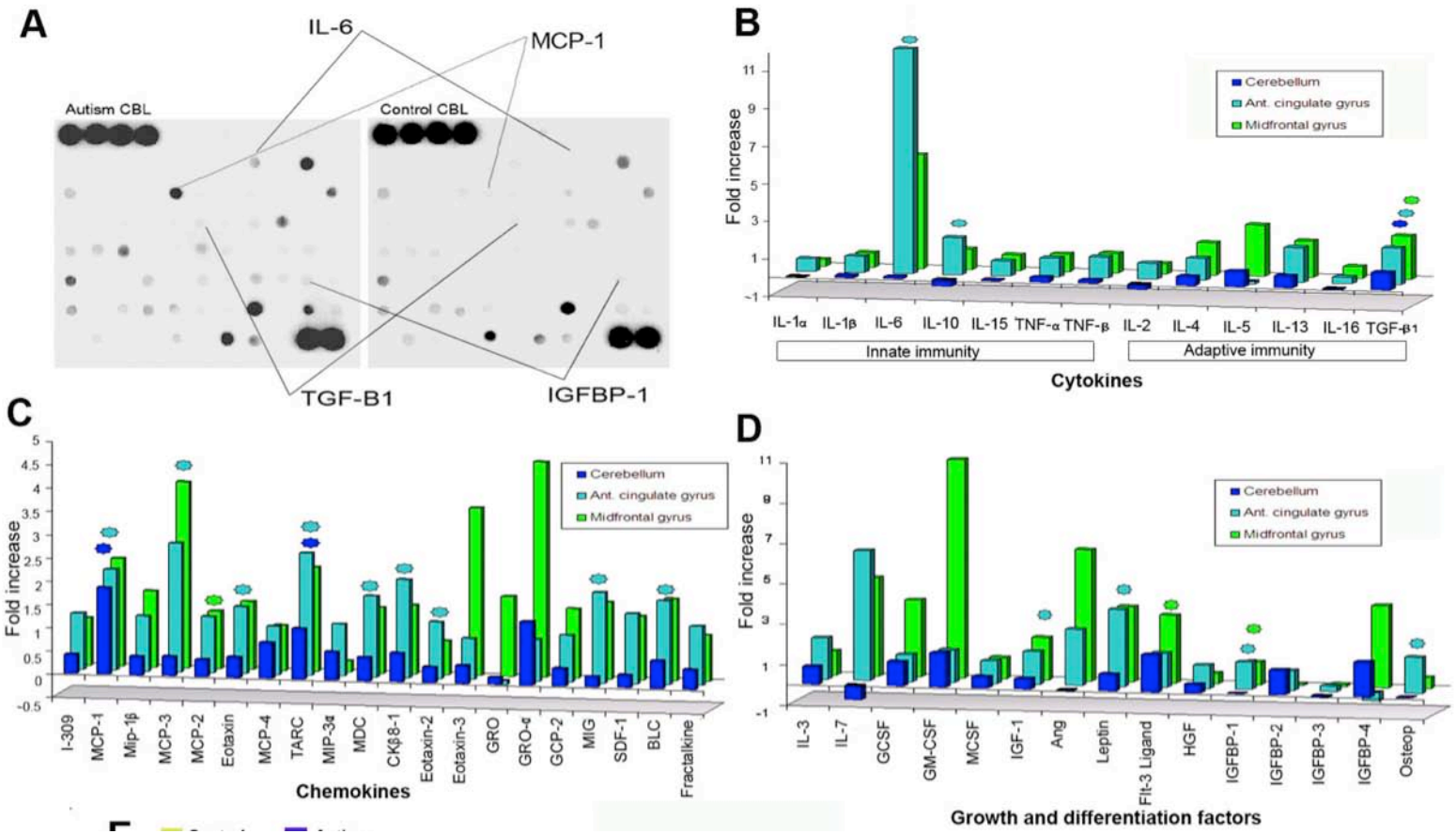






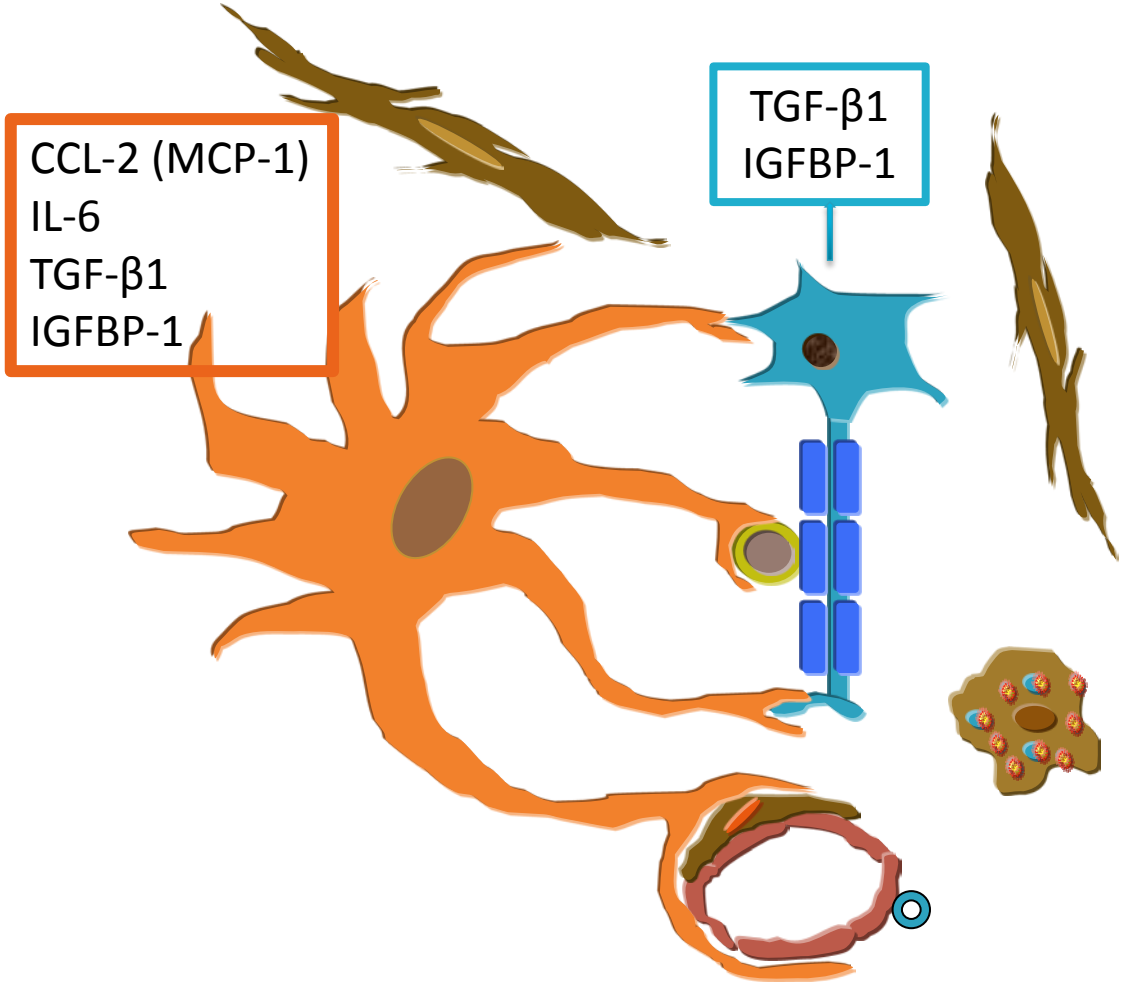
Morgan JT et al. Biological Psychiatry 2010

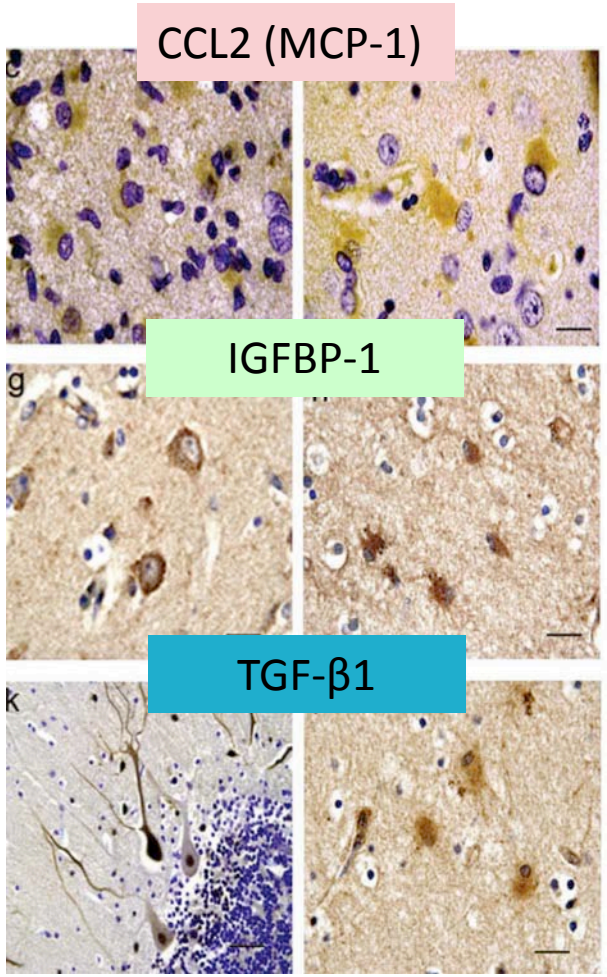
Autism: Profiles of Cytokine/Chemokine in the Brain



Vargas DL et al. Ann Neurol 2005

Brain Cytokine-Chemokine-Growth Factors in ASD





Based on Vargas DL et al. Ann Neurol 2005

Transcriptomic analysis of autistic brain reveals convergent molecular pathology

Irina Voineagu[1], Xinchen Wang[2], Patrick Johnston[3], Jennifer K. Lowe[1], Yuan Tian[1], Steve Horvath[4], Jonathan Mill[3], Rita M. Cantor[4], Benjamin J. Blencowe[2] & Daniel H. Geschwind[1,4]

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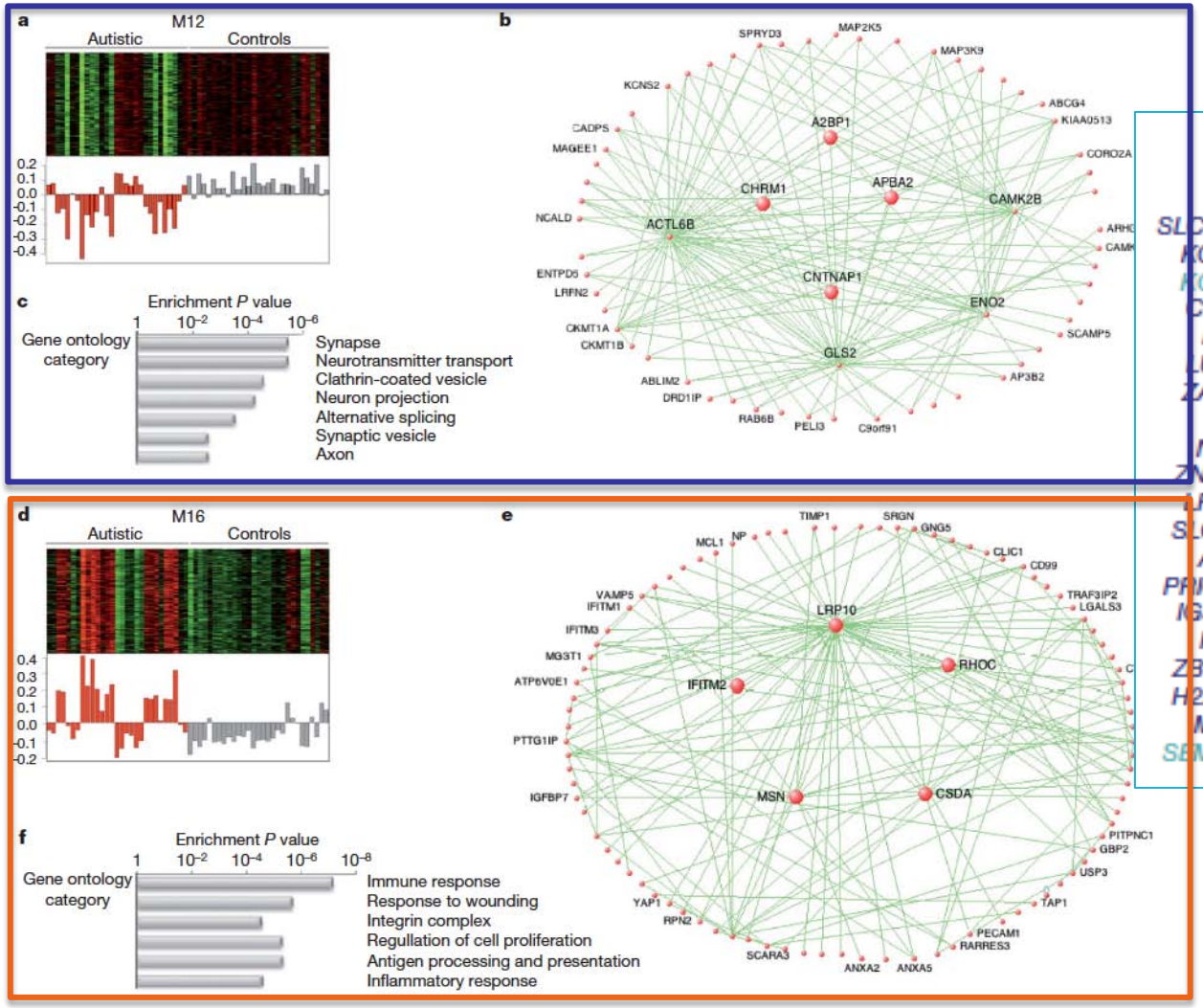
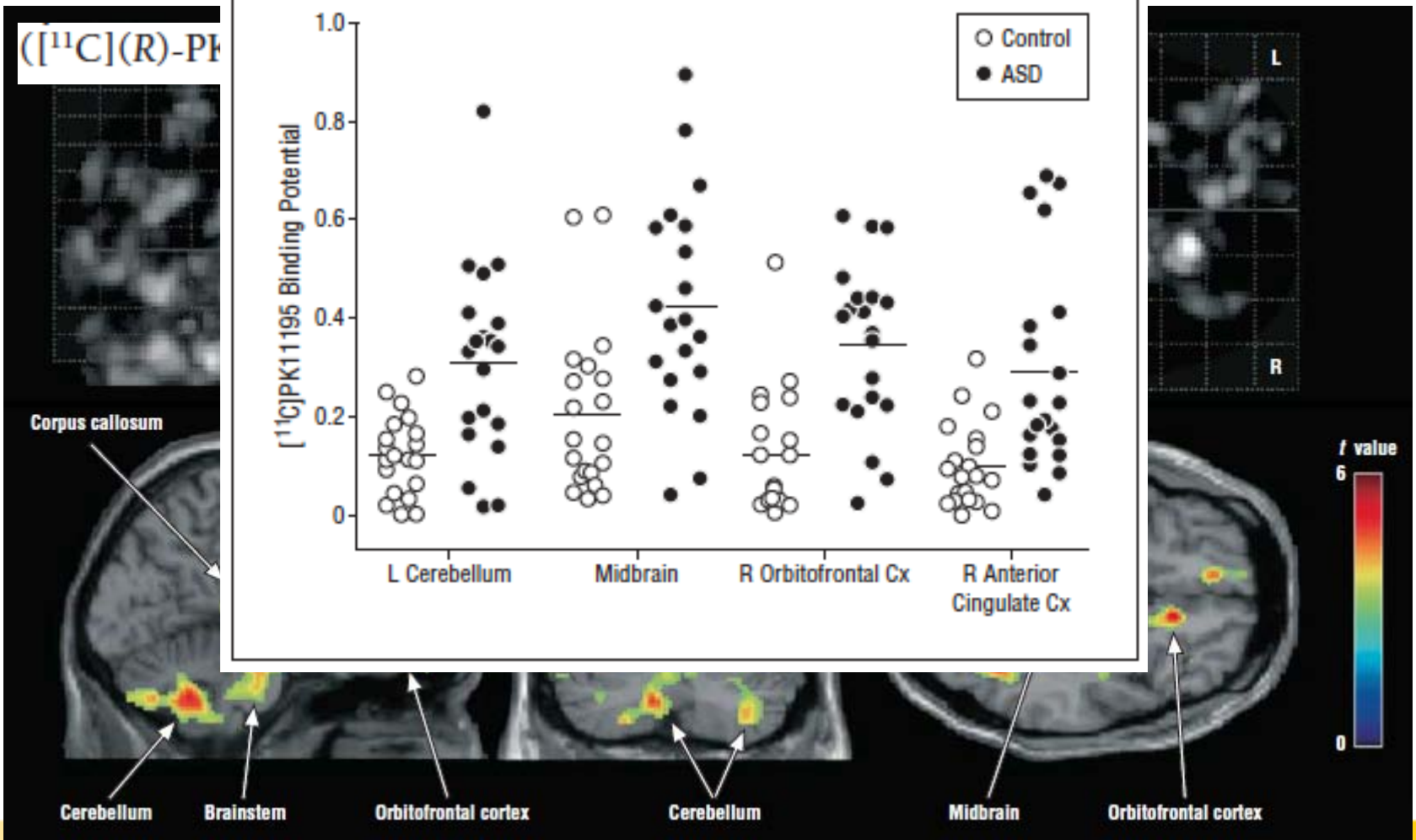


ONLINE FIRST

Microglial Activation in Young Adults With Autism Spectrum Disorder

Katsuaki Suzuki, MD, PhD; Genichi Sugihara, MD, PhD; Yasuomi Ouchi, MD, PhD; Kazuhiko Nakamura, MD, PhD; Masami Futatsubashi, BS; Kiyokazu Takebayashi, MD, PhD; Yujiro Yoshihara, MD, PhD; Kei Omata, PhD; Kaori Matsumoto, MA; Kenji J. Tsuchiya, MD, PhD; Yasuhide Iwata, MD, PhD; Masatsugu Tsujii, MA; Toshirou Sugiyama, MD, PhD; Norio Mori, MD, PhD

Arch Gen Psychiatry. ...ber 26, 2012.



Neuroinflammatory responses in the brain in ASD: what means as co-morbidity ?

The current evidence:

- **Increased innate immunity responses in the brain**
  - Microglial & astroglial responses
  - Cytokine & chemokine increases or disregulation
- **Differential transcriptome expression for synaptic and immune related genes**
- **In-vivo evidence of microglial "activation" (PET scanning)**

Trigger
?

❑ ***Brain pathology***

Abnormal brain development?
Response to intrauterine injury ?
(e.g. infection, maternal antibodies)
Abnormal cortical function ?
(e.g. epilepsy)

❑ ***Peripheral Immune responses?***

Host autoimmunity?
Systemic inflammation?
Microbial translocation?

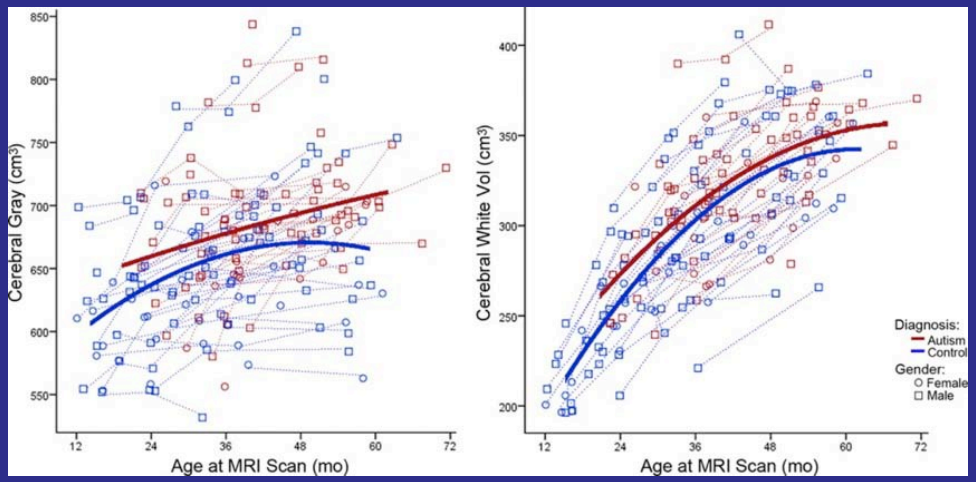
What are the triggering factors in neuroinflammation in the brain of ASD patients ?

Observations in the neuropathology of ASD:

Excess neurons, white matter and enlarged frontal lobe

What is abnormal in the white matter?

Filipek et al. 1991
Piven et al.1995,1996
Courchesne E et al. 2001-2004
Sparks et al. 2002
Aylward et al 2002
Herbert et al 2003-2005
Hardan A, 2006
Others…

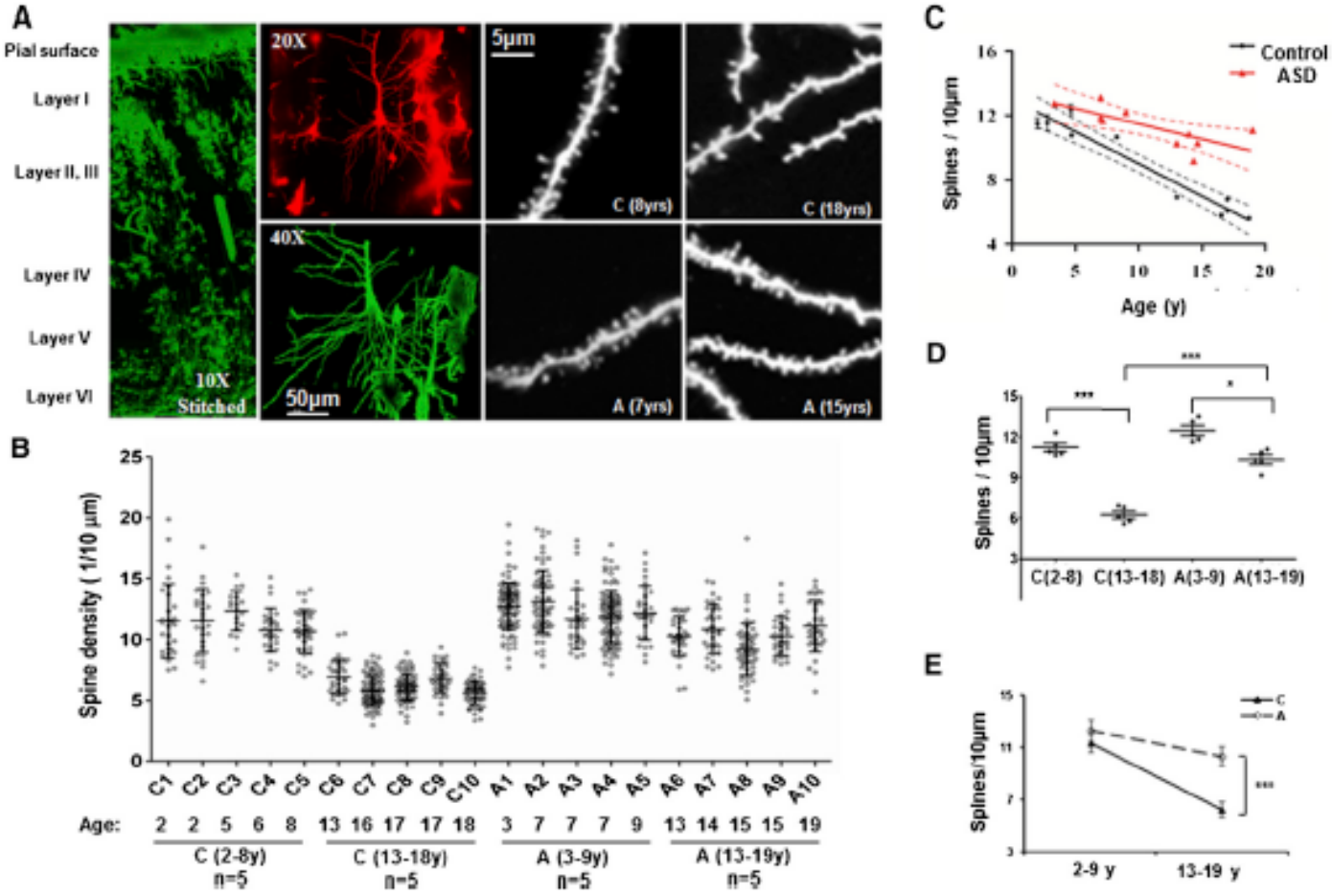


Schumann CM,
J Neurosci 2010

Loss of mTOR-Dependent Macroautophagy Causes Autistic-like Synaptic Pruning Deficits

Guomei Tang,[1] Kathryn Gudsnuk,[2] Sheng-Han Kuo,[1] Marisa L. Cotrina,[3,7] Gorazd Rosoklija,[4,8] Alexander Sosunov,[3] Mark S. Sonders,[1] Ellen Kanter,[1] Candace Castagna,[1] Ai Yamamoto,[1] Zhenyu Yue,[6] Ottavio Arancio,[3] Bradley S. Peterson,[4,8] Frances Champagne,[2] Andrew J. Dwork,[3,4,8] James Goldman,[3] and David Sulzer[1,4,5,8,*]

Neuron 83, 1131–1143, September 3, 2014



Microglia Sculpt Postnatal Neural Circuits in an Activity and Complement-Dependent Manner

Dorothy P. Schafer,[1] Emily K. Lehrman,[1,5] Amanda G. Kautzman,[1,5] Ryuta Koyama,[1] Alan R. Mardinly,[3] Ryo Yamasaki,[4] Richard M. Ransohoff,[4] Michael E. Greenberg,[3] Ben A. Barres,[2] and Beth Stevens[1,*]

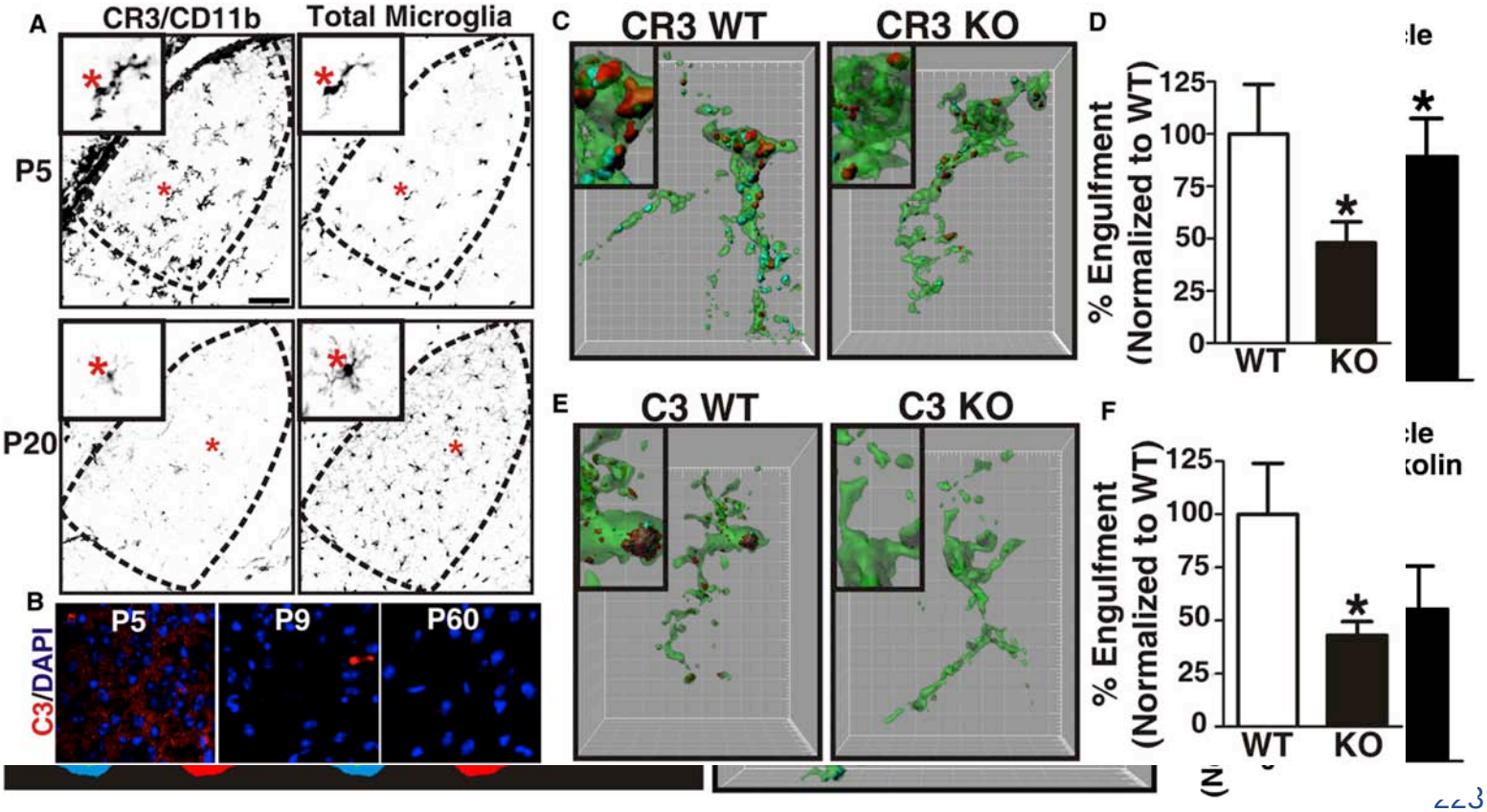
[1]Department of Neurology, F.M. Kirby Neurobiology Center, Children's Hospital, Harvard Medical School, Boston, MA 02115, USA

[2]Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305, USA

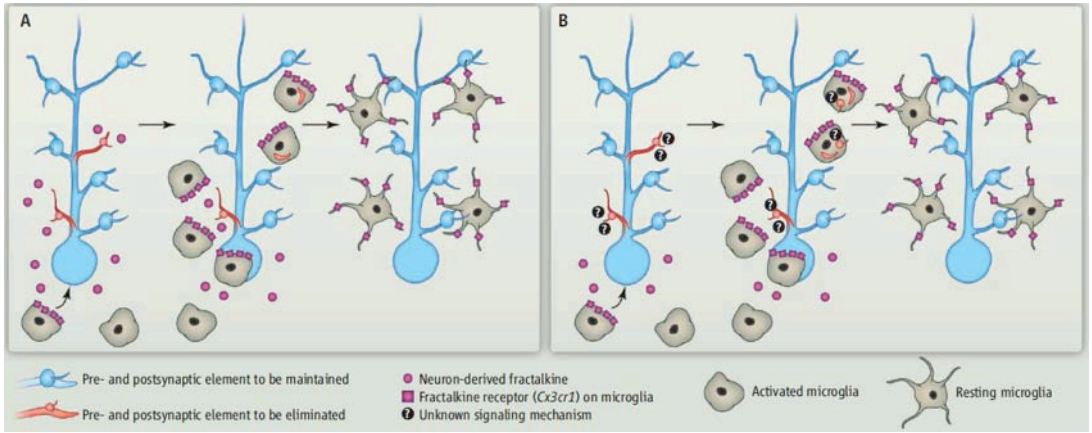
[3]Department of Neurobiology, Harvard Medical School, Boston, MA 02115, USA

[4]Neuroinflammation Research Center, Department of Neurosciences, Lerner Research Institute, and Mellen Center for MS Treatment and Research, Neurological Institute, Cleveland Clinic, Cleveland, OH 44195, USA

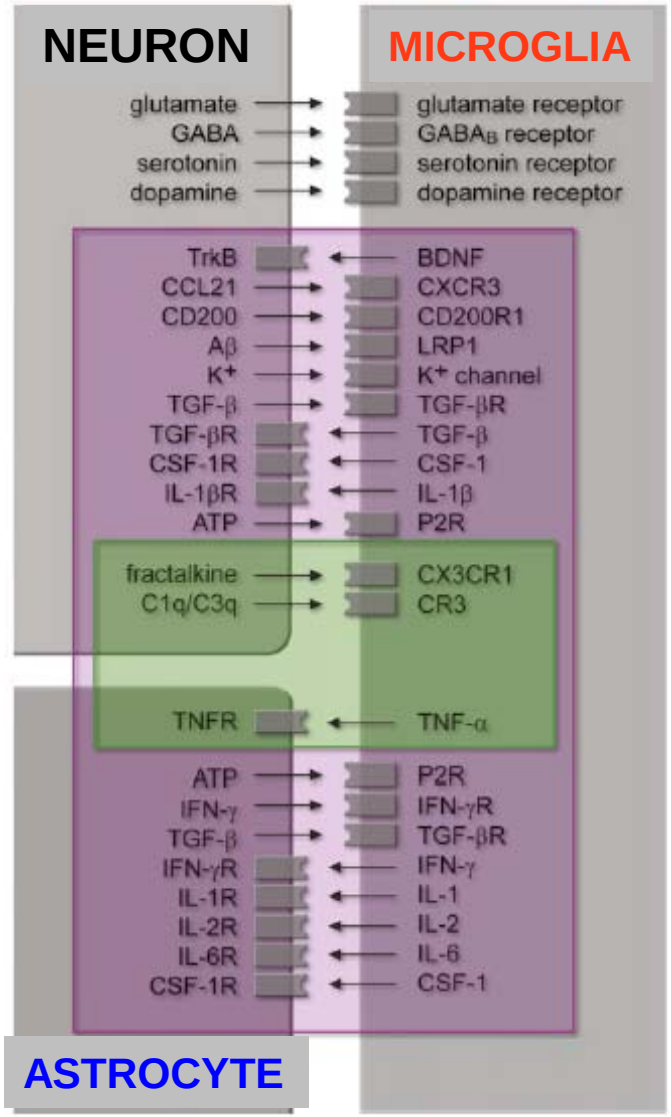
Neuron 74: 691-705, 2012



Microglia is a critical cell from the innate immune system for modeling neuronal networks and circuits during development and adaptive brain plasticity

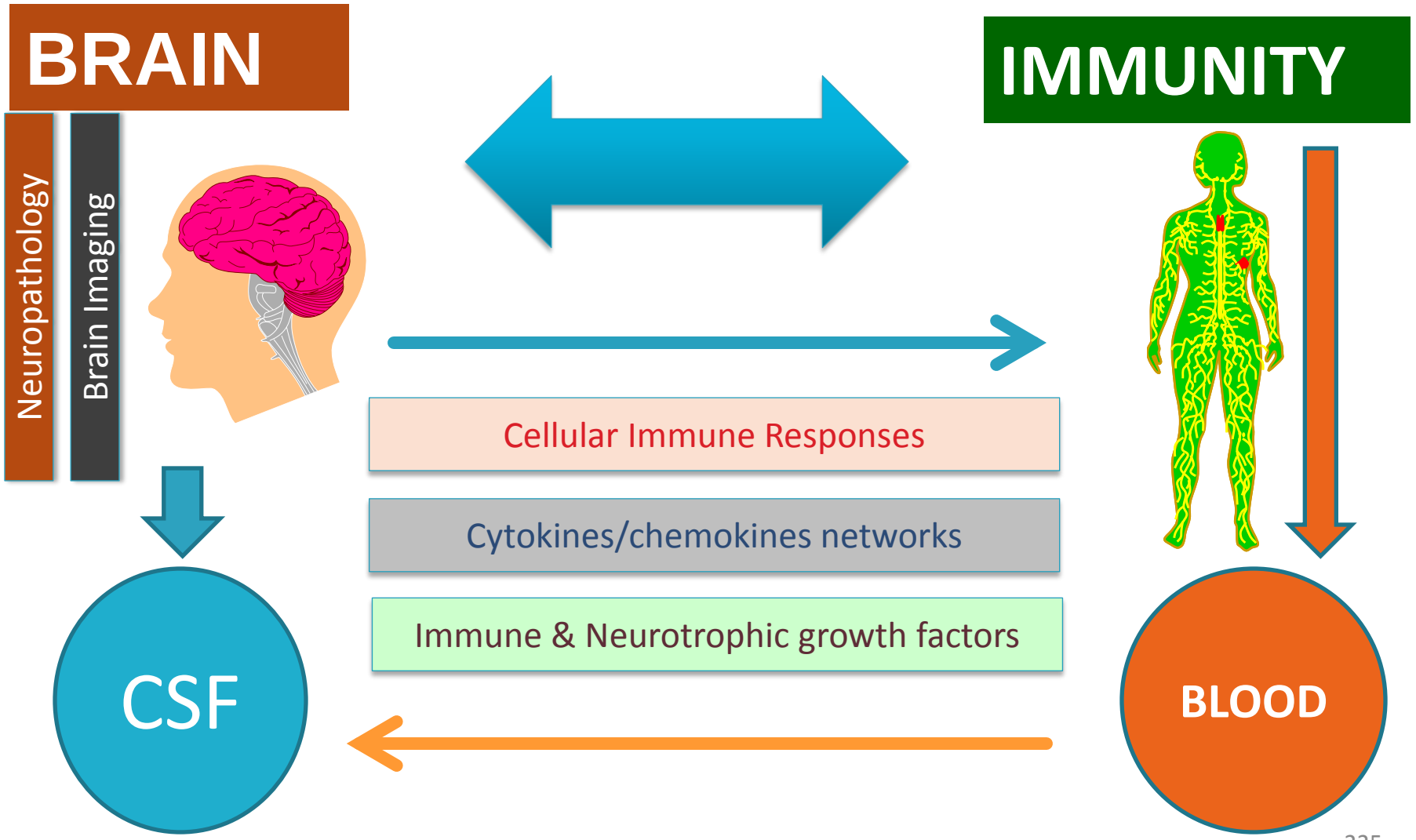


How Many Cell Types Does It Take to Wire a Brain?
Richard M. Ransohoff and Beth Stevens
Science **333**, 1391 (2011);



Neuronal-Microglia-Astrocyte Interactions. Kettenmann H, Neuron 2103

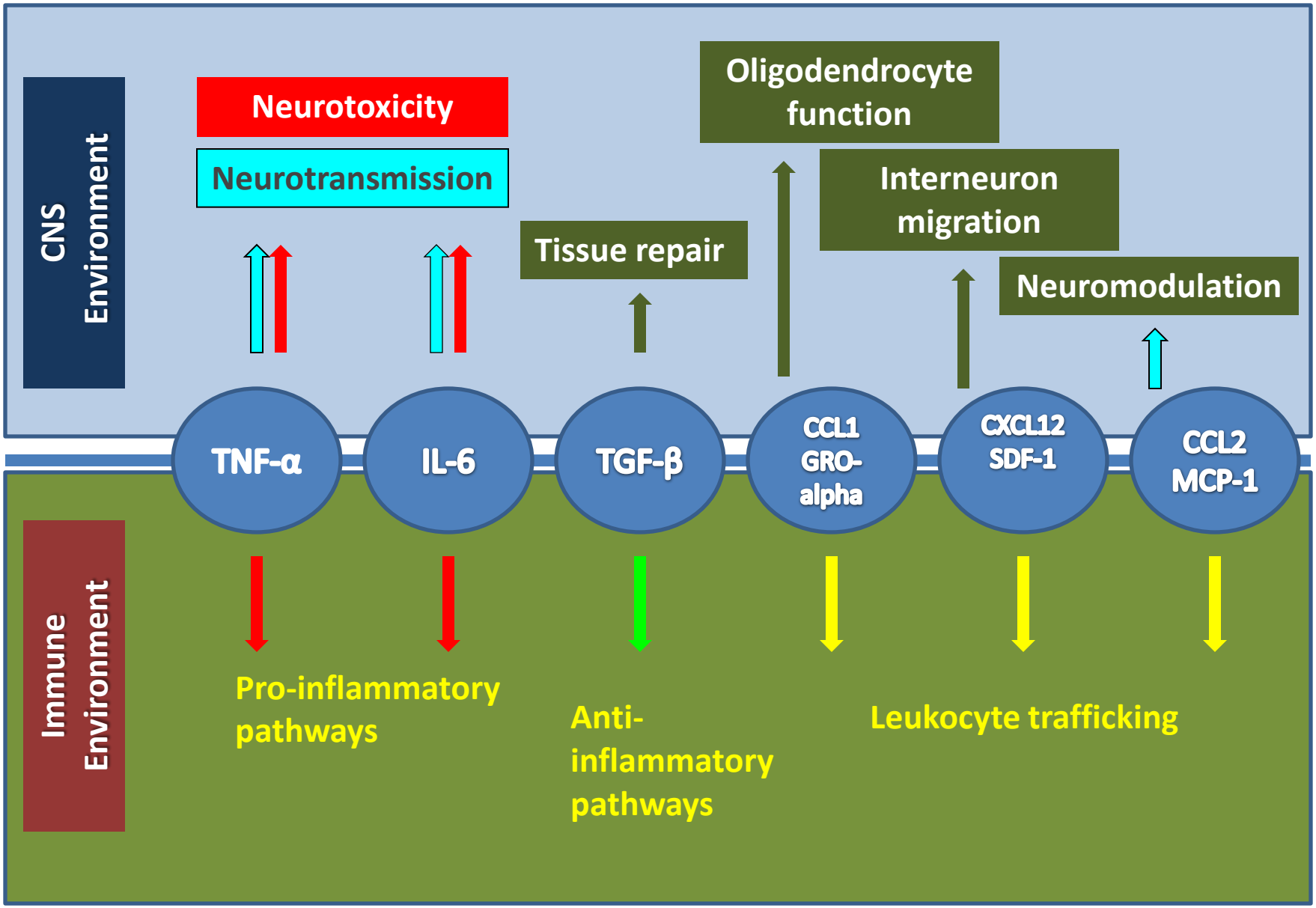
HOW TO STUDY NEURAL AND IMMUNE INTERACTIONS IN ASD?

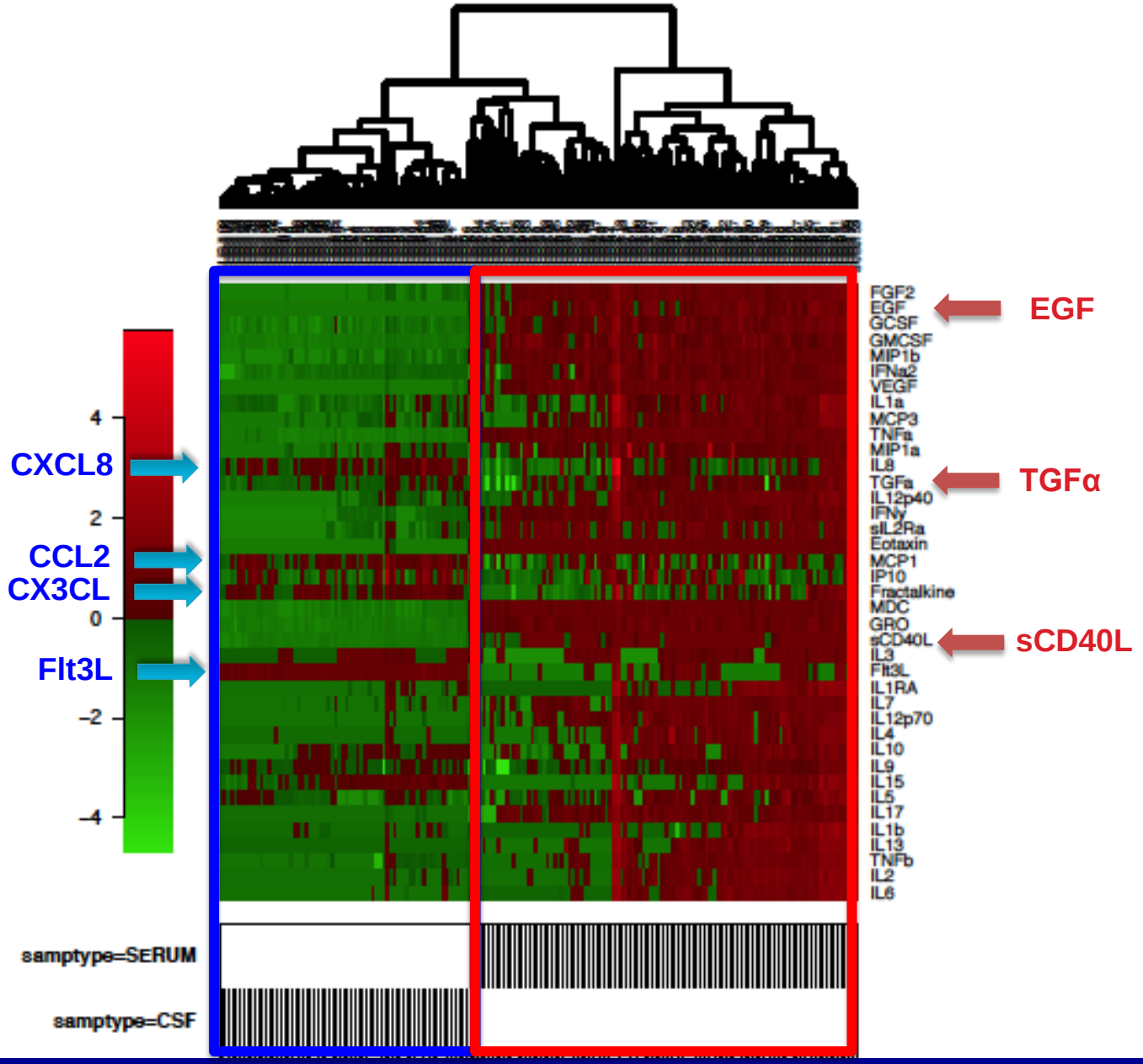


Autism Intramural Program

S. Swedo, A. Thurm & others

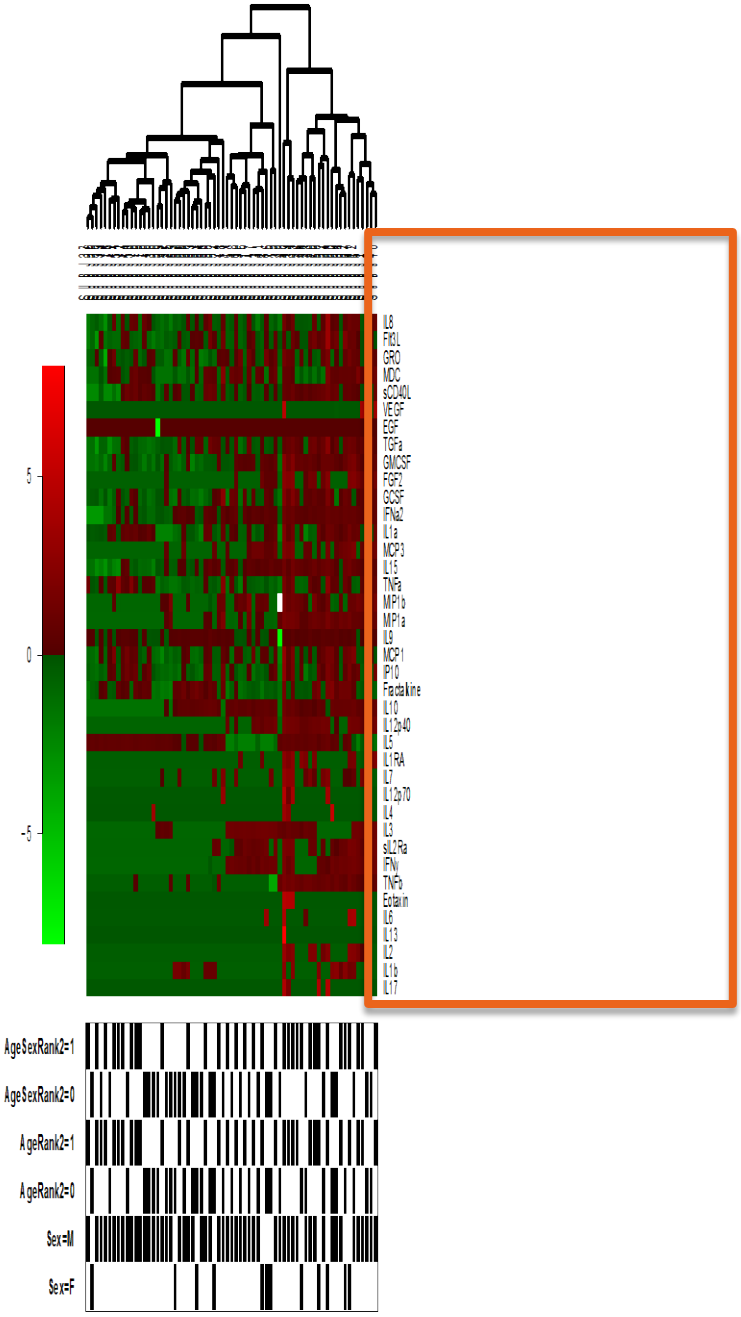
- Prospective study of patients with ASD
  - Non-regressive vs. “regressive”
  - Clinical, behavioral and cognitive assessment
  - Neuroimaging
  - **Blood/CSF studies for evaluation of immune activation**

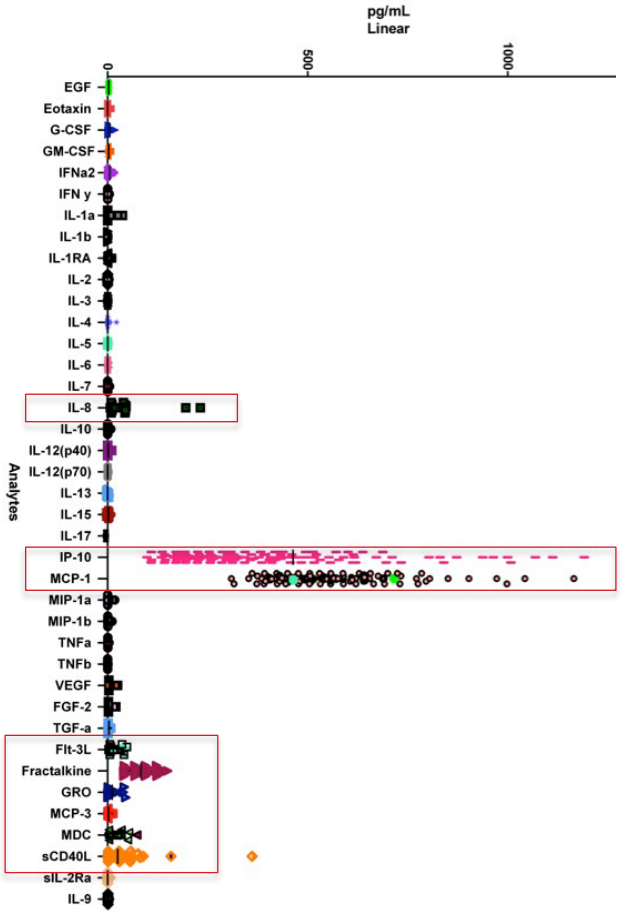

CNS Environment
Neurotoxicity
Neurotransmission
Tissue repair
Oligodendrocyte function
Interneuron migration
Neuromodulation
TNF-α
IL-6
TGF-β
CCL1 GRO-alpha
CXCL12 SDF-1
CCL2 MCP-1
Immune Environment
Pro-inflammatory pathways
Anti-inflammatory pathways
Leukocyte trafficking



NIMH Study of Immunological Factors in Autism:

Profile of cytokines and chemokines in serum & CSF





Prevalence of Immune Related Proteins in the CSF of patients with ASD

Autism and the Immune Response

What we know:

- Various immune system abnormalities have been reported in children with autistic disorders by a number of different laboratories.
- Both enhanced autoimmunity and reduced immune function have been shown.
- Development of ‘autism’ animal models with immune basis

Studies on Plasma cytokines

Study Description	Reference
Elevated levels of IL-1b, IL-6, IL-8 and IL-12p40. Associated with regression	(Ashwood, et al., 2011b)
Increase in chemokine **MCP-1, Rantes** and **Eotaxin** levels in ASD subjects compared to age-matched typically developing controls. An association between increases chemokines levels with aberrant behaviors.	(Ashwood et al., 2011c)
In male ASD subjects, an increase in cytokines **IL-1beta, IL-1RA, IL-5, IL-8, IL-12(p70), IL-13, IL-17** and **GRO-alpha.**	(Suzuki et al., 2011)
Increase in **leptin** levels in ASD subjects compared to age-matched controls.	(Ashwood et al., 2008b)
Increase in **macrophage migration inhibitory factor (MIF)** in ASD subjects compared to age-matched controls.	(Grigorenko et al., 2008)
Decrease in **TGF-beta** in subjects with ASD compared to controls.	(Ashwood et al., 2008a; Okada et al., 2007)
Increase in **IL-12** and **IFN-gamma** in ASD subjects compared to age-matched controls.	(Singh, 1996)

Serum Cytokines/Chemokines

Studies in chronological order	Immune Measure	Behavior Measure
Ashwood et al. (2008)	Low serum levels of TGFβ1	Stereotypy, Irritabilty, Lethargy, and Hyperactivity
Grigorenko et al. (2008)	High serum levels of MIF	Impaired Sociability
Kajizuka et al. (2010)	High serum levels of PDGF	Stereotypy
Ashwood et al. (2011a)	High serum chemokines CCL2/MCP-1, CCL5/RANTES and eotaxin	Lethargy, Stereotypy, Hyperactivity, Impaired Communication and Socialization
Ashwood et al. (2011b)	High serum levels of cytokines IL-1β, IL-6, IL-8 and IL-12p40	Lethargy, Stereotypy, and Hyperactivity, and Impaired Communication

Cytokine/Chemokines- activated cells

Study Description	Reference
In isolated PBMCs stimulated with PHA, increase in **GM-CSF, TNF-alpha** and **IL-13**. A decrease in **IL-12(p40)** in ASD subjects vs. controls.	(Ashwood et al., 2011d)
Stimulation of TLR on monocytes - ASD vs. to age-matched controls. Increase in **IL-1beta, IL-6, TNF-alpha**, with stimulation of TLR2. Increase in **IL-1beta**, with stimulation of TLR4. Decrease in **IL-1beta, IL-6, GMCSF, TNF-alpha** with TLR9.	(Enstrom et al., 2010)
Increase in **IFN-gamma** in NK cells from subjects with ASD.	(Enstrom et al., 2009b)
Increase production of cytokines from Th1 and Th2 cytokines in ASD subjects vs age-matched controls.	(Molloy et al., 2006)
Increase in **IL-12** and **TNF-alpha** in ASD subject with GI symptoms.	(Jyonouchi et al., 2005)
Increase in **IFN-gamma** and **TNF-alpha** in isolated PBMCs from ASD subjects compared to age-matched controls stimulated with LPS.	(Jyonouchi et al., 2002)
Unstimulated whole blood from ASD vs. age-matched controls – increase in **IFN-gamma** and **IL-1RA** with -higher **IL-6** and **TNF-alpha.**	(Croonenberghs et al., 2002)
Unstimulated PBMC- ASD subjects: higher levels of **TNF-alpha, IL-1beta,** and **IL-6** vs. controls. PBMCs stimulated with LPS, PHA and tetanus produced increase levels of **IL-12** and **IL-1beta**.	(Jyonouchi et al., 2002)

Dynamic Cellular Responses

Studies in chronological order	Immune Measure	Behavior Measure
Onore et al. (2009)	PHA induced IL-23 production	Impaired Sociability
Enstrom et al. (2010)	High LPS induced IL-1B production in purified monocytes	Imparied Sociability and Communication
Ashwood et al. (2011)	Higher T lymphocyte production of IFNγ, IL-8, TNFα lower IL-13, IL-10, IL-5	Hyperactivity, Stereoptypy, Lethargy, and Impaired Communication and Sociability
Han et al. (2011)	Higher T lymphocyte, cytotoxic lymphocyte and total lymphocyte numbers	Stereotypy, executive function
Breece et al. (2012)	Increased frequency of myeloid dendritic cells	Stereotypy, Regression, GI symptoms, amygdala volume

Immune dysregulation in ASD- evidence from response to environmental toxicant

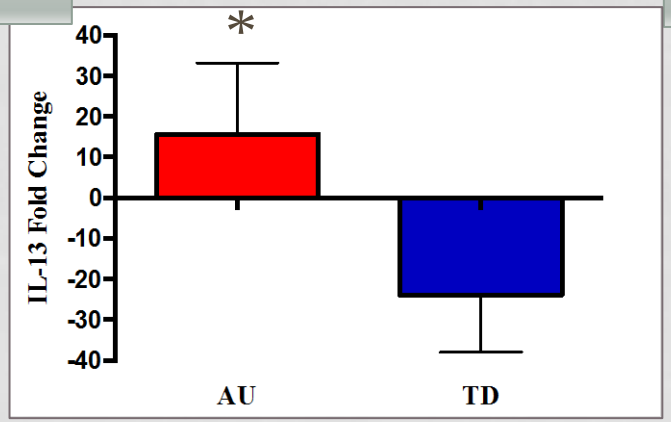
- **Differential Immune response in the presence of the toxicant BDE-49**
- ASD vs. TD Subjects demonstrate opposite response to co-culture with polybrominated diphenyl ether (BDE)-49
- Is this an example of genetic susceptibility for immune dysregulation in the context of environment?

Non-activated immune cells- Differential response to BDE-49

250nM BDE-49

IL-13

Th2 Cytokine



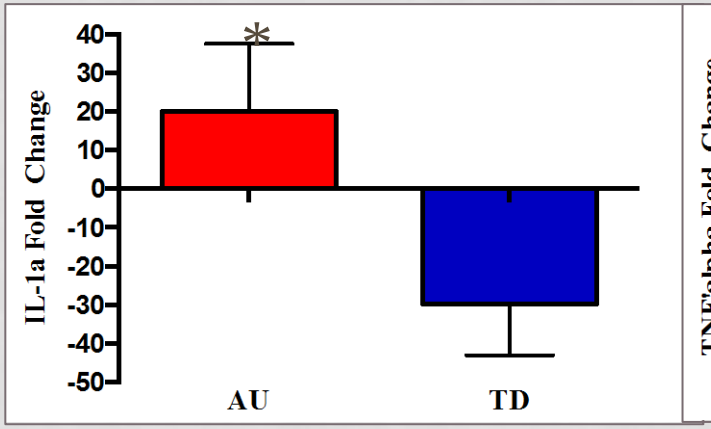
50nM BDE-49

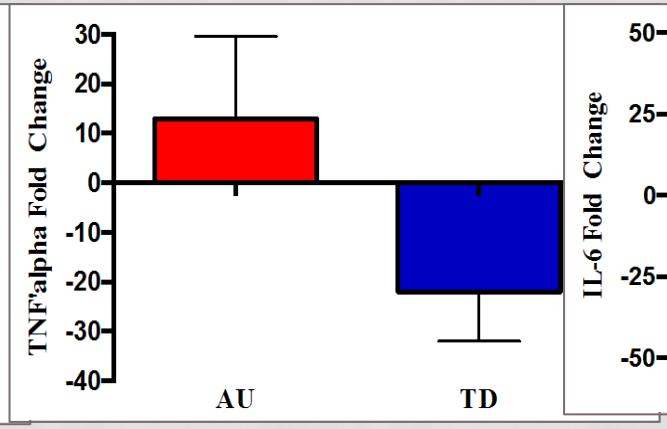
Inflammatory cytokines

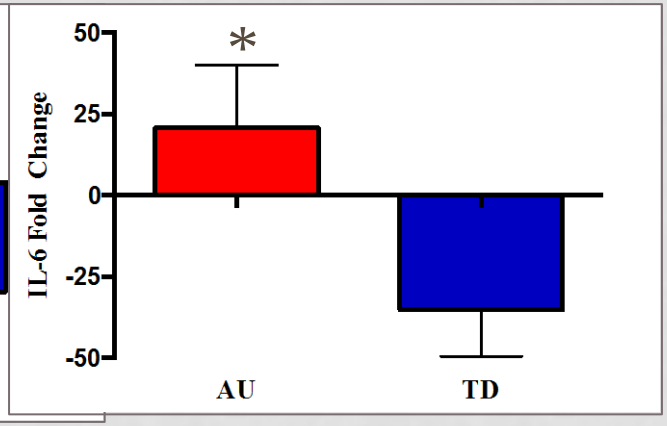
IL-1α

TNFα

IL-6





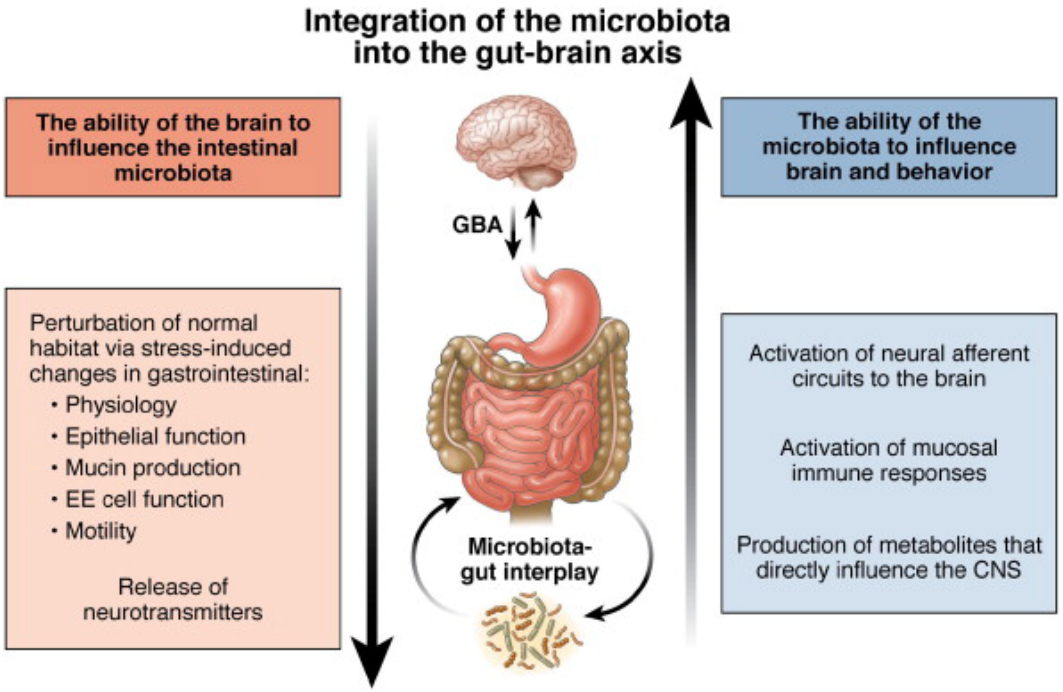


Mean fold change was derived from $\log_n$ transformed pg/ml cytokine production (Treatment-Baseline/Baseline). (t-test,*p=<0.05)

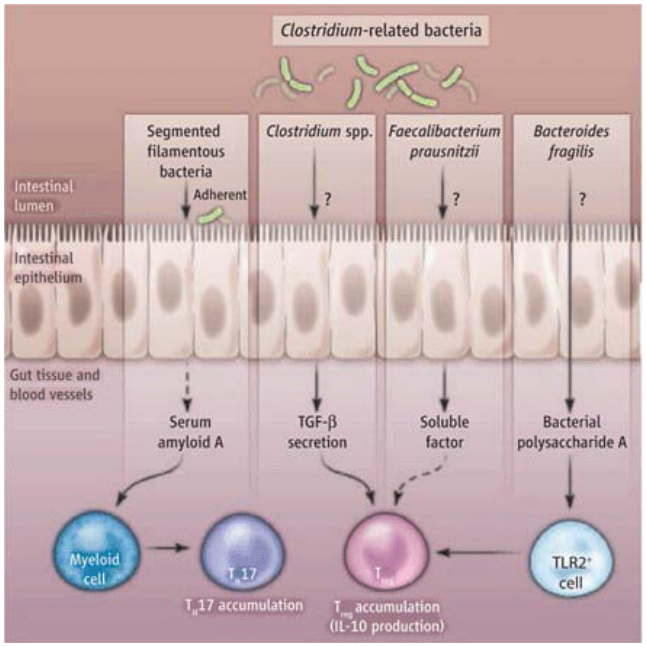
Are there systemic factors such as GI pathology involved in ASD immune abnormalities?

The Gut Microbiota Influence the CNS Function

The Gut Microbiota Modulate the Immune System

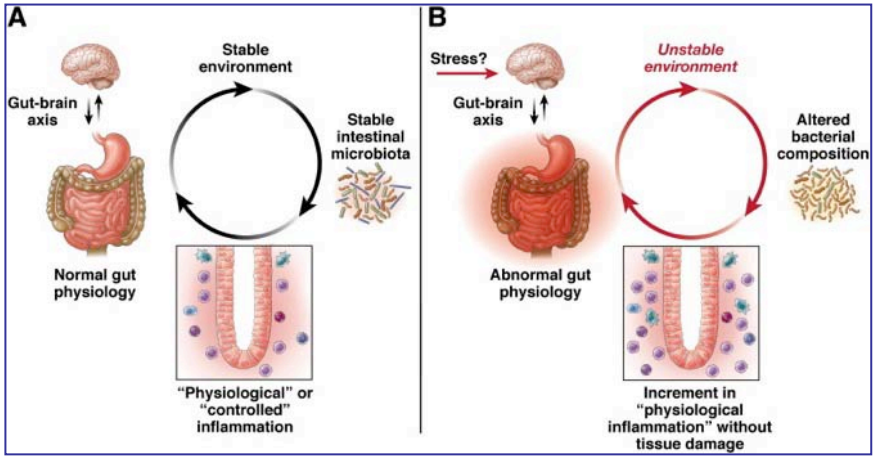


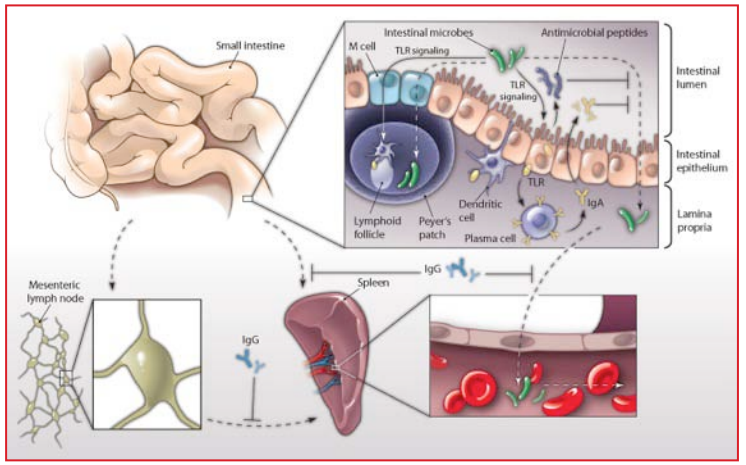
Collins SM & Bercik P.
Gastroenterology136, 2009: 2003-2014



M J Barnes, F Powrie
Science 2011;331:289-290

Collins SM & Bercik P. Gastroenterology 2009: 2003-2014

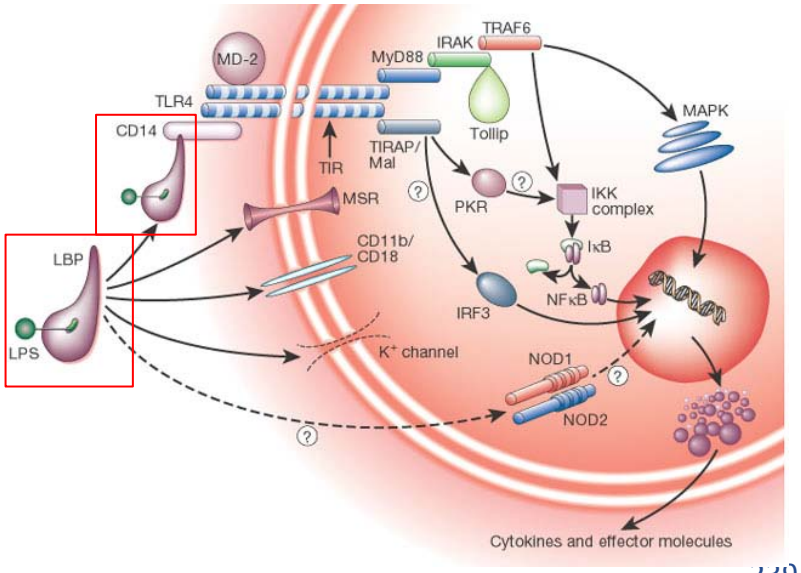




Microbial Translocation:

The innate and adaptive immune systems coordinate containment of intestinal microbes

Lipopolysacharide (LPS), a component of Gram negative bacteria cell walls, **LPS-binding protein (LBP)**, and **anti-endotoxin core immunoglobulin** levels have been shown to be reliable biomarkers of MT along with markers of immune activation such as sCD14 and proinflammatory cytokines .



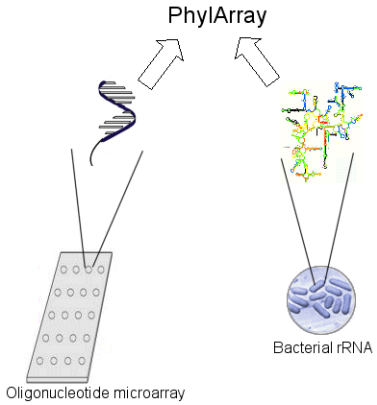
D. A. Hill et al., Sci. Signal. 2, pe77 (2009)

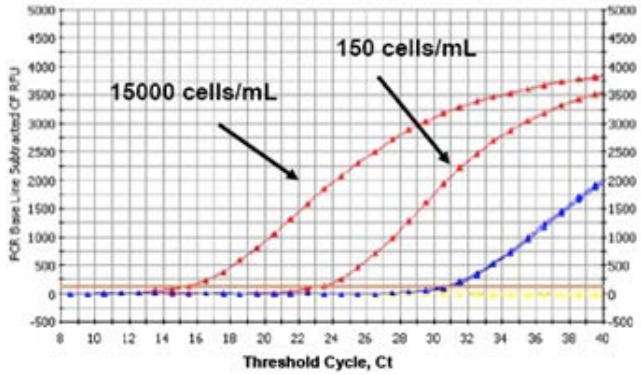
MT markers in ASD

Subjects	LPS (mean rank)	LBP (mean rank)	IgM (mean rank)	IgG (mean rank)
Autism (n=57)	42.56	40.57	45.27	43.40
Typical (n=33)	50.58	51.38	45.89	49.12
P	**0.161**	**0.056**	**0.913**	**0.418**

Subjects	LPS (mean rank)	LBP (mean rank)	IgM (mean rank)	IgG (mean rank)
Autism – Regressors (n=23)	29.46	26.36	29.79	32.84
Autism- Non Regressors (n=34)	28.91	31.57	27.83	23.33
P	**0.974**	**0.240**	**0.661**	**0.034***

Analysis of the 16S rRNA in plasma of ASD vs. control patients





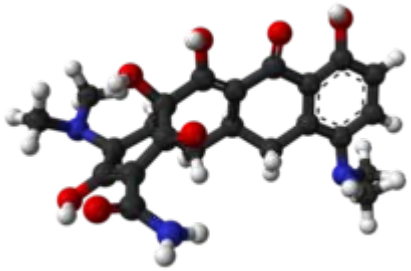
	Group	Mean	St.Dev.	N	Sig
Ct1	Autism	27.87	1.92	55	
	Typical	27.47	1.80	34	.183
Ct2	Autism	27.29	2.01	55	
	Typical	27.00	1.81	34	.366
Ct3	Autism	27.53	1.98	55	
	Typical	27.2	1.61	34	.366

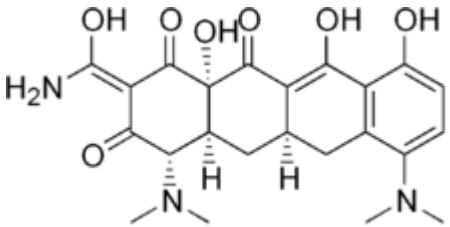
MT in ASD: Conclusions

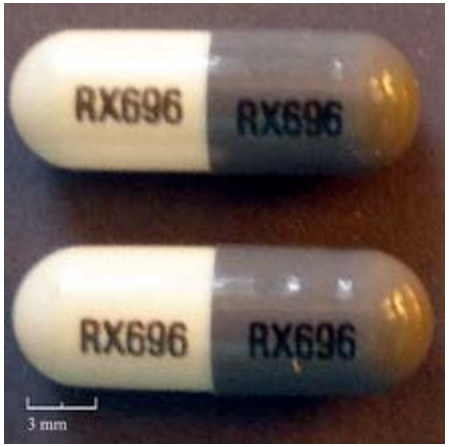
Circulating levels of MT markers, LPS, LPB, or anti-endotoxins, ***did not differ significantly*** between children with autism and age-matched typical controls, nor did a history of regression correspond to evidence of circulating MT markers.

There is not a valid rationale for the empiric use of antibiotics in the treatment of hypothetical "leaky gut" or "occult" infections in children with autism.

How to modify microglial activation?







- **Minocycline hydrochloride**, is a broad spectrum tetracycline antibiotic.
- It is the most lipid-soluble of the tetracycline-class antibiotics with greatest penetration into brain.
- Minocycline has been used in many neurological disorders:

Neuroprotective and anti-human immunodeficiency virus activity of minocycline. Zink MC, et al. JAMA. 2005; 293(16):2003-11.

Minocycline attenuates HIV infection and reactivation by suppressing cellular activation in human CD4+ T cells. Szeto GL, et al J Infect Dis. 2010;201:1132-40

Minocycline slows disease progression in a mouse model of amyotrophic lateral sclerosis. Kriz J, Nguyen MD, Julien JP. Neurobiol Dis. 2002 ;10:268-78.

Placebo-controlled phase I/II studies of minocycline in amyotrophic lateral sclerosis. Gordon PH, et al. Neurology. 2004; 62:1845-7.

Efficacy of minocycline in patients with amyotrophic lateral sclerosis: a phase III randomised trial. Gordon PH, and the Western ALS Study Group. Lancet Neurol. 2007 (12):1045-53.

Pardo et al. Journal of Neurodevelopmental Disorders 2013, 5:9
http://www.jneurodevdisorders.com/content/5/1/9

RESEARCH Open Access

A pilot open-label trial of minocycline in patients with autism and regressive features

Carlos A Pardo[1*], Ashura Buckley[2], Audrey Thurm[2], Li-Ching Lee[3], Arun Azhagiri[1], David M Neville[2] and Susan E Swedo[2]

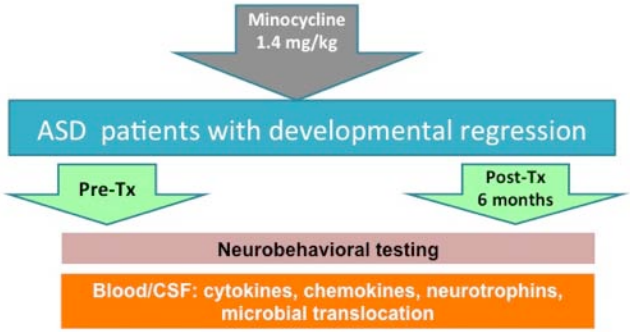


Table 1 Patient sample description

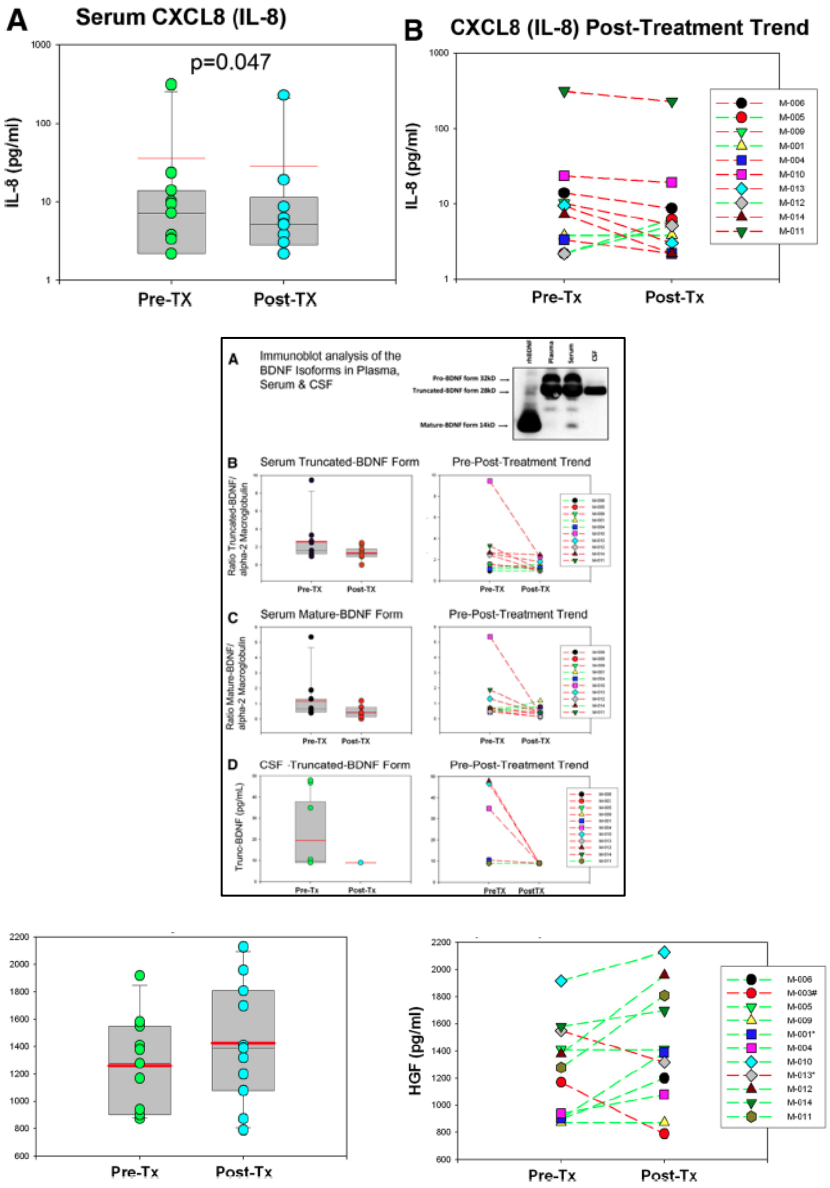
Subject	Pretreatment age (months)	Sex	Age at onset of regression (months)	Clinical assessment				
				Non-verbal IQ/DQ	CGI- baseline	CGI- post-treatment	Vineland- ABC baseline	Vineland-ABC post-treatment
1	99	F	27	30	6	5	55	54
2[a]	64	M	13	60	5	4[a]	68	73[a]
3	69	M	12	33	5	5	45	44
4	153	M	19	77	5	5	56	55
5	91	M	8	58	3	3	60	56
6	66	M	18	103	4	4	70	71
7	128	F	15	25	5	5	55	46
8	112	M	14	33	5	5	53	56
9	49	M	18	59	4	4	66	63
10	38	M	22	90	4	3	70	66
11	83	M	18	32	5	5	48	57
				M ± *SD*	4.6 ± 0.8	4.4 ± 0.8	58.7 ± 8.8	58.3 ± 9.3
				Effect Size	-	−0.3	-	−0.1

[a]Denotes child that discontinued the study at 3 months.
Note: Effect size was calculated for the within-subjects effect using the following formula: $ES = (M_{Post} - M_{BL})/SD_{BL}$). CGI, Clinical Global Impression.

Table 3 Treatment effect on cytokines, chemokines, metalloproteinases and growth factors[a]

Analyte	Cerebrospinal fluid (CSF)		Serum		Plasma	
	Z	P	Z	P	Z	P
TNFα	−0.45	0.66	−1.78	0.074		
IL-6	−0.36	0.72	−1.75	0.08		
CCL2 (MCP-1)	−1.78	0.075	−1.27	0.24		
CCL3 (MIP-1α)	−0.18	0.86	−0.76	0.45		
CCL5 (RANTES)	−0.73	0.47	0.66	0.61		
CXCL8 (IL-8)	−0.45	0.66	−1.99	**0.047**		
BDNF[b]	−2.03	**0.042**			−0.76	0.45
Truncated-BDNF/α-2 M[c]			−2.19	**0.028**		
Mature-BDNF/α-2 M[c]			−0.87	0.386		
CD40L	−0.89	0.37			−1.33	0.18
GDNF	−0.37	0.71			−1.83	0.07
HGF	−2.19	**0.028**			−1.25	0.21
Leptin	−1.48	0.14			−0.27	0.79
MMP-1					−1.07	0.29
MMP-3					−0.15	0.88
MMP-7					−1.89	0.05
MMP-8					−0.05	0.96
MMP-9					−1.38	0.17

[a]Selected analytes, Mann–Whitney *U*-test.
[b]Quantified by Luminex technique. In CSF only the truncated-BDNF form appears to be detectable in immunobotting experiments.
[c]Quantified by immunoblotting and densitometry analysis. Significance was calculated based on ratio BDNF isoform/α-2 M.



Pardo *et al. Journal of Neurodevelopmental Disorders* 2013, **5**:9

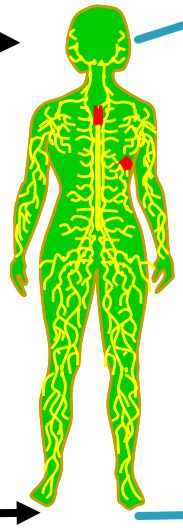
IMMUNOTHERAPIES IN AUTISM: Are these good options??

Minocycline, COX2 inhibitors , NSAIDs

IV Immunoglobulins (IVIG)

Immunomodulators
e.g. Steroids
(eg. Prednisone)

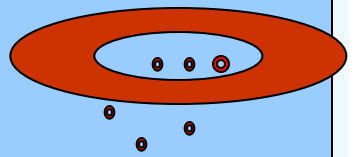
Immunosupressants
e.g. Cyclophosphamide
Methotrexate,
tacrolymus, etc



CNS:

- **Innate immunity**
  Microglia, astroglia, cytokines, chemokines

BBB



- **Adaptive immunity**
  Specific T cell and antibody responses

Peripheral Immune System

Brain NeuroImmunity

Autism and the Immune Response Overall Conclusions

- Various neuroimmune system abnormalities have been reported in children with autistic disorders:
  - Neuroglial and innate immune responses may reflect homeostatic mechanisms rather than pathogenic immunological reactions
- Maternal antibodies have been demonstrated in subsets of mother of children with autism, a finding that suggest a potential role of maternal-immune mediated mechanisms in neurodevelopmental disarrangements

Autism and the Immune Response Overall Conclusions

- Systemic immune abnormalities such as variable expression of cytokines and chemokines, increase of NK cells and decreased immunoglobulin G levels may reflect a dysfunctional immune response rather than an autoimmune or immunologically pathogenic process.
- This dysregulation may inform as to dysfunction in pathways common to both the immune and nervous systems.
- Gastrointestinal symptoms are not associated with microbial translocation or represent inducers of immune activation.
- **There is no role at this time for the use of immunological therapies, immmunosupressants or antibiotics to "modify" neurological or behavioral abnormalities in autism.**