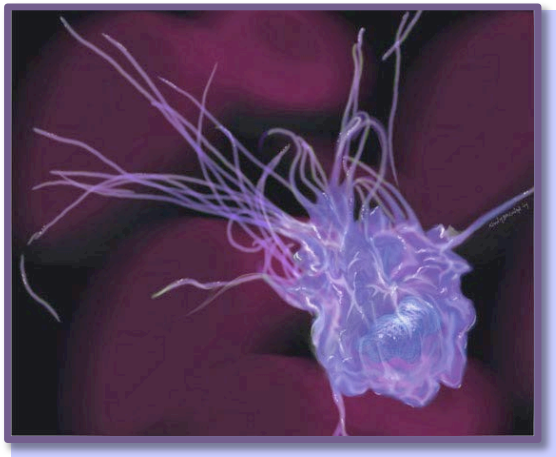
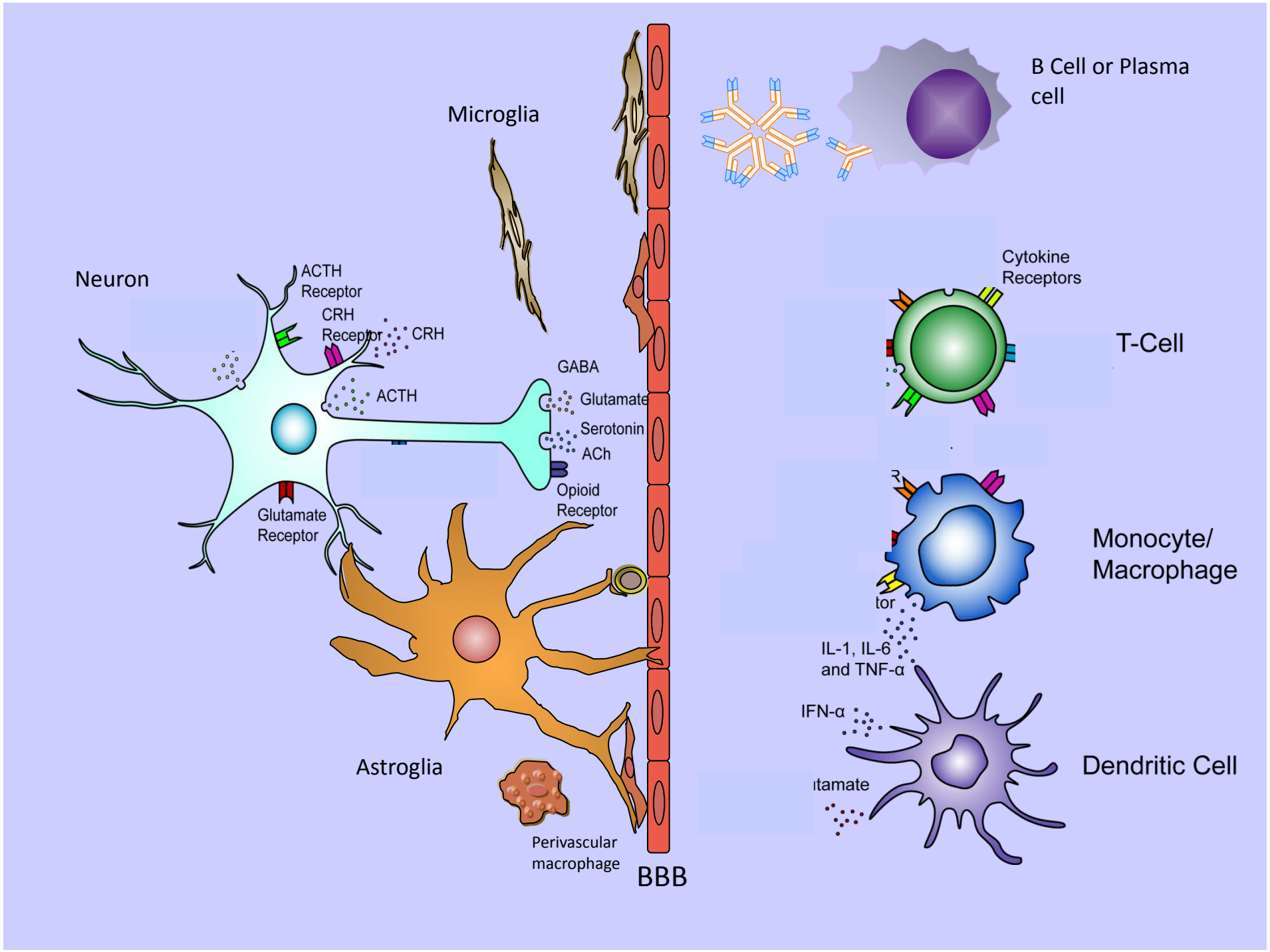


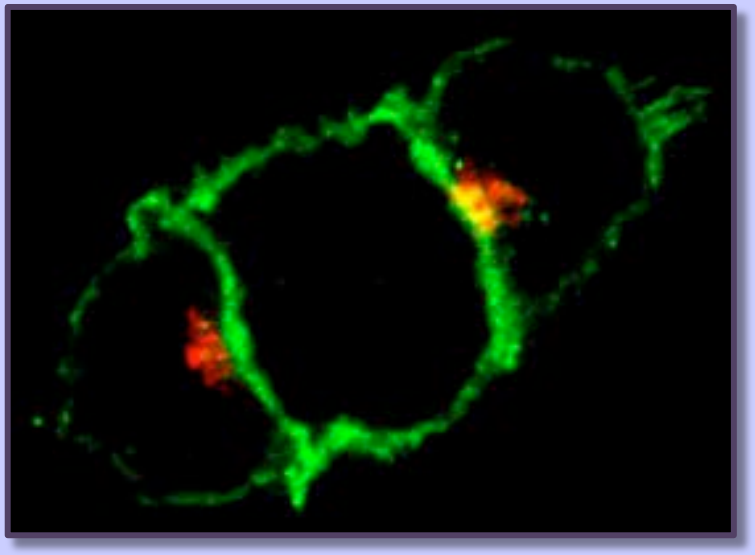
Immunologic Factors in the Etiology of Autism

Judy Van de Water, Ph.D.
Professor, Department of Internal Medicine
University of California, Davis
UC Davis M.I.N.D. Institute
javandewater@ucdavis.edu

Neuro-immune interactions

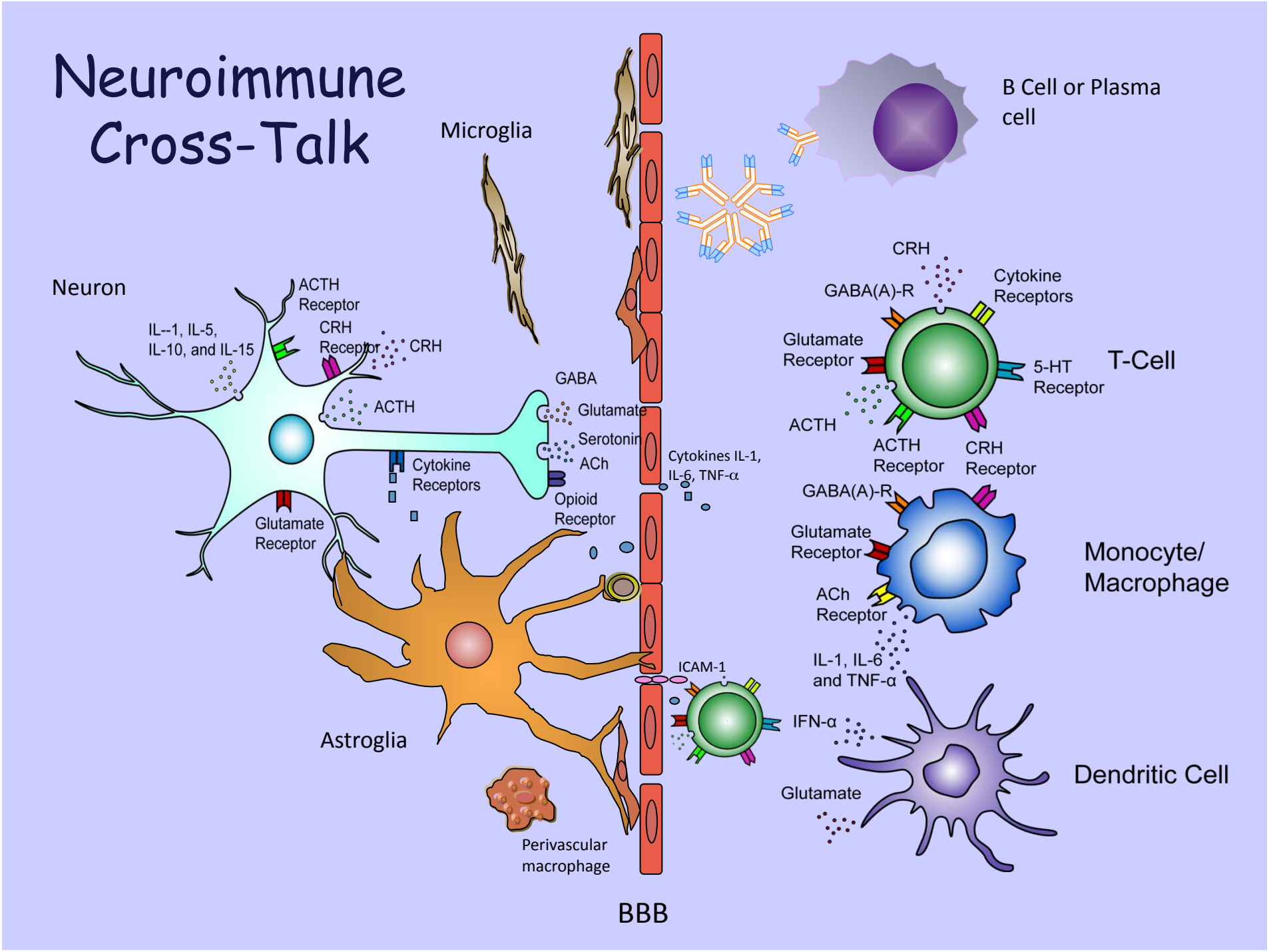
- For the past 60 years, the central nervous system has been considered to be immune privileged.
- The classic paradigm was there is interaction between these two seemingly distinct systems only during disease.


Microglia
B Cell or Plasma cell
Neuron
ACTH Receptor
CRH Receptor
CRH
ACTH
GABA
Glutamate
Serotonin
ACh
Opioid Receptor
Glutamate Receptor
Cytokine Receptors
T-Cell
Monocyte/ Macrophage
IL-1, IL-6 and TNF-α
IFN-α
Dendritic Cell
Astroglia
Perivascular macrophage
BBB



Neuro-immune interactions

- Results from diverse fields now show clear and convincing evidence of bidirectional communication between the nervous and immune systems


Neuroimmune Cross-Talk
Microglia
B Cell or Plasma cell
Neuron
ACTH Receptor
IL--1, IL-5, IL-10, and IL-15
CRH Receptor
CRH
ACTH
GABA
Glutamate
Serotonin
ACh
Cytokine Receptors
Opioid Receptor
Glutamate Receptor
Cytokines IL-1, IL-6, TNF-α
CRH
GABA(A)-R
Cytokine Receptors
Glutamate Receptor
5-HT Receptor
T-Cell
ACTH
ACTH Receptor
CRH Receptor
GABA(A)-R
Glutamate Receptor
ACh Receptor
Monocyte/ Macrophage
IL-1, IL-6 and TNF-α
ICAM-1
IFN-α
Glutamate
Dendritic Cell
Astroglia
Perivascular macrophage
BBB

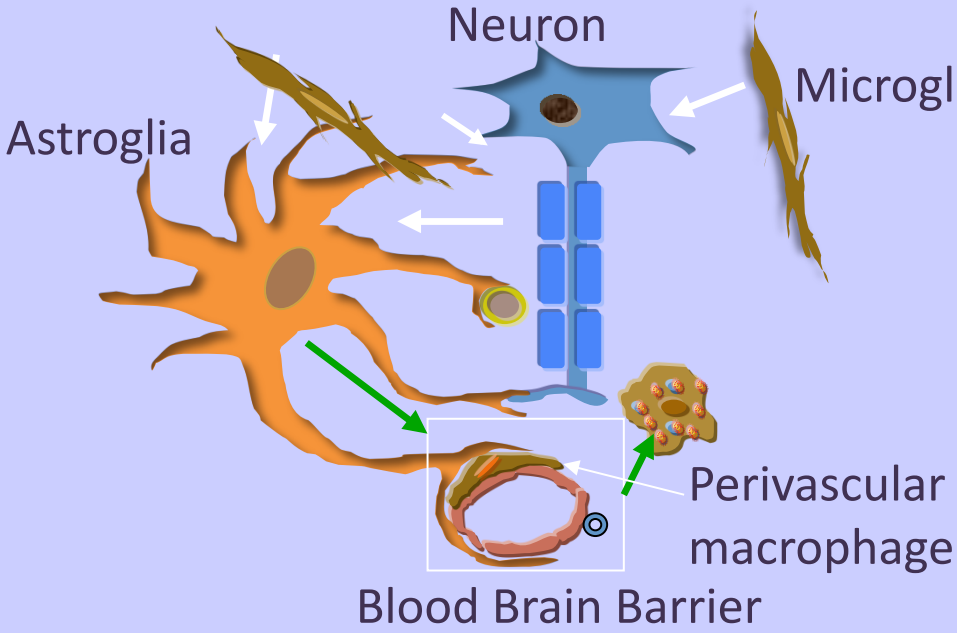
The health and development of the nervous system is largely dependent upon the health and function of the immune system

What are the factors that influence this cross-talk?

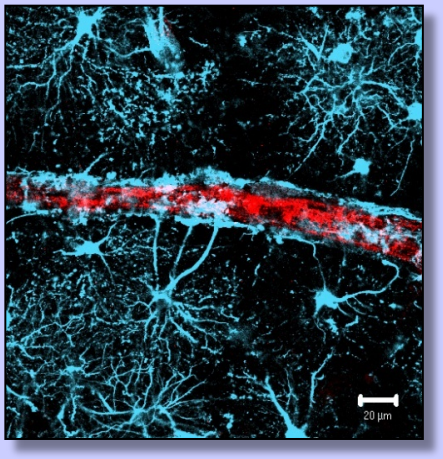
Cross-talk between the Immune and Central Nervous Systems

- Communication between the cells of the immune and nervous systems is possible on several levels.
- Neurons, microglia, and astrocytes can produce cytokines and express cytokine receptors.
- This enables them to direct immune cells as well as respond to various immunological stimuli.
- Cells of the immune system are able to secrete various neurotransmitters and express receptors for many of these molecules.
- This allows immune cells to impact neural processes and respond to neural stimuli.

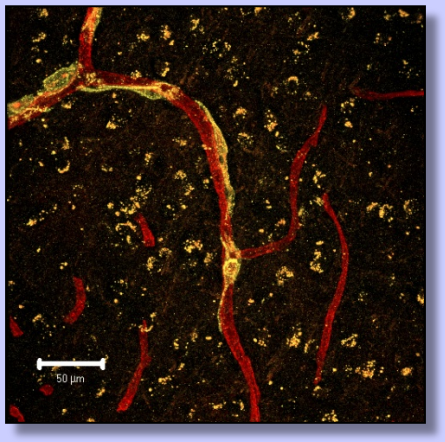
The neuroimmune system is comprised of different "immune" cells and governed by different mechanisms



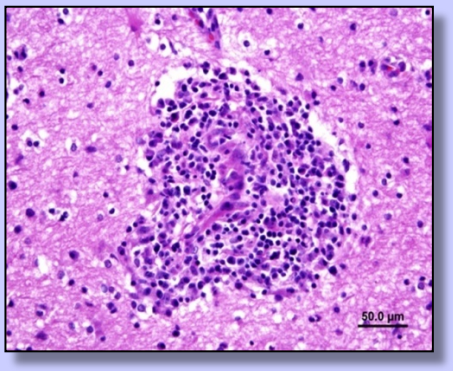
Perivascular microglia



Perivascular astrocytes



Perivascular infiltrate



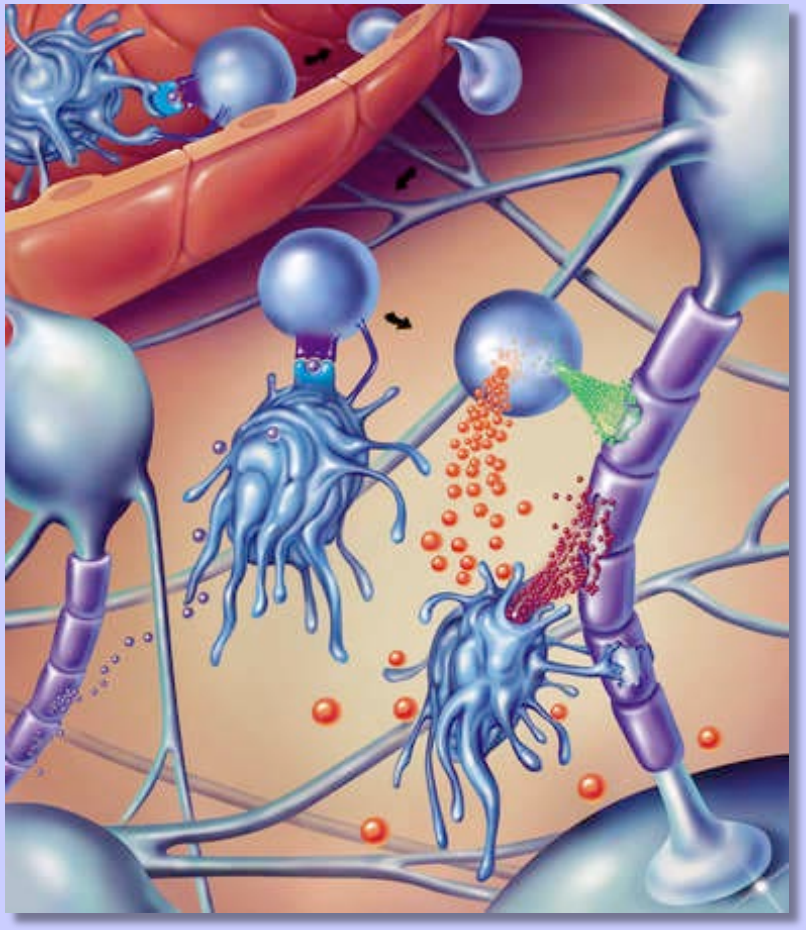
Modulation of immune responses
Lymphocyte/monocyte trafficking

C.Pardo

What happens in the developing brain?

We are a long way from knowing everything about the neuro-immune interface during development.

Immune and Nervous System Interactions

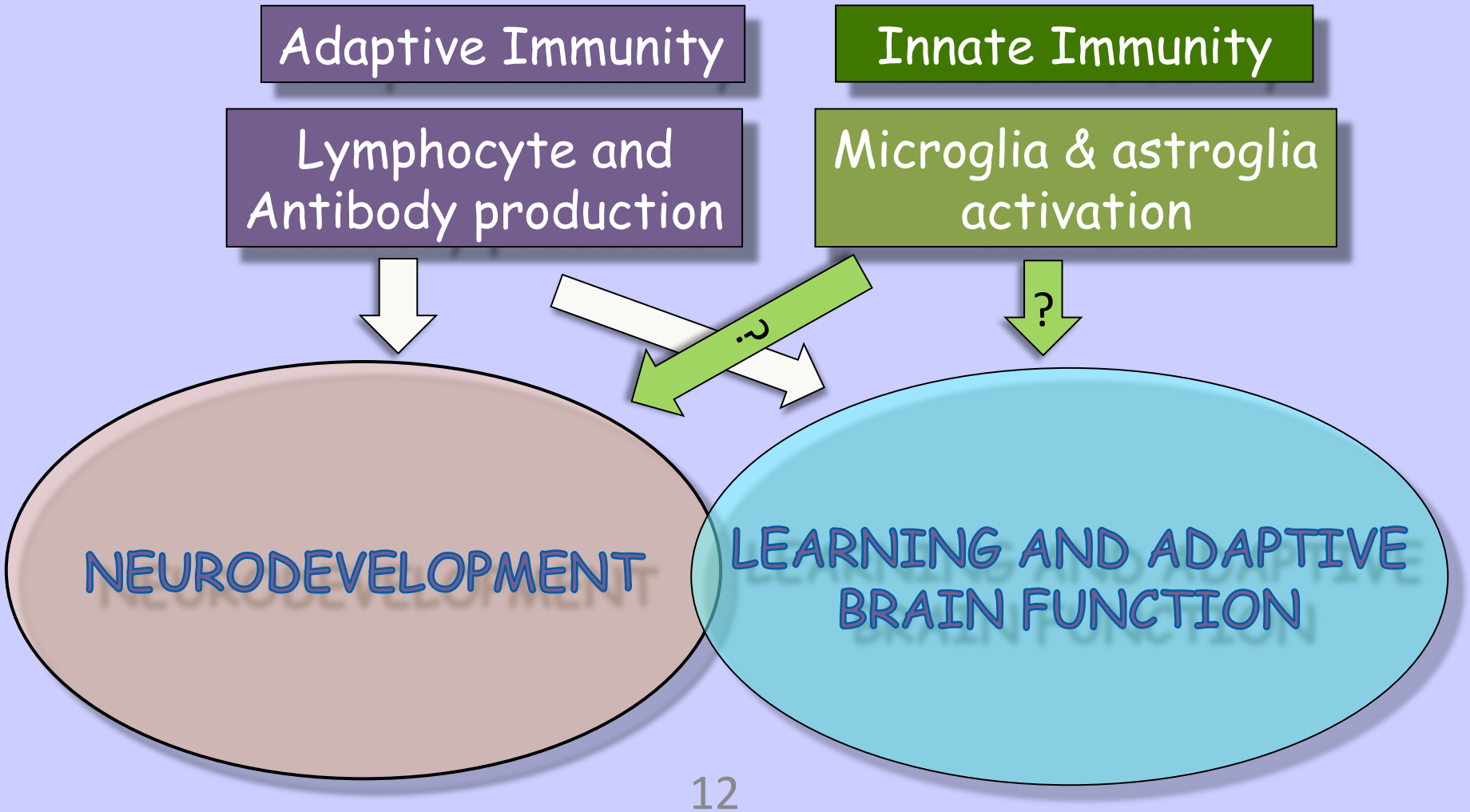


- An altered immune response may impact other biological systems including the neuroendocrine and nervous systems, and vice versa.

The neuroimmune interface is critical during gestation and the early post-natal period

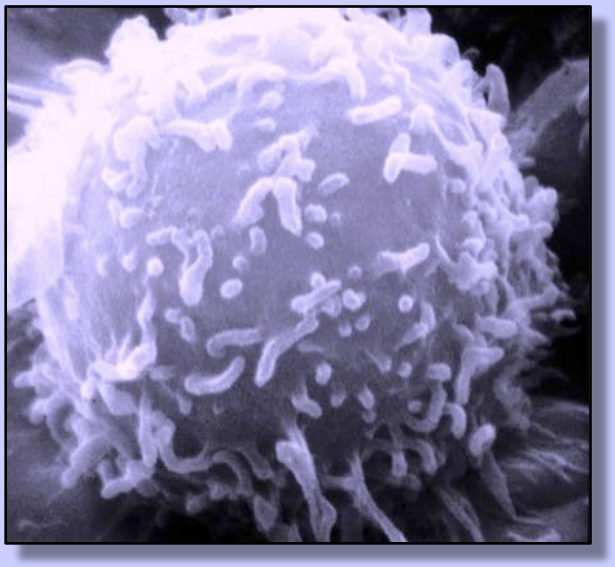
- Data suggests that altered cytokine profiles affect neurodevelopmental outcome
  - Both schizophrenia and autism are thought to result in part from changes in the maternal immune profile during gestation.
- These changes include increased inflammatory cytokines and autoantibodies to proteins in the developing fetal brain.

Are neuroimmune mechanisms involved in pathogenesis of ASD?



What is the status of the cellular immune response?

- To analyze this, we look at cytokine levels in the blood, and in culture supernatants after stimulation.

Activation →  → Cytokine production

What are cytokines? Molecules that tell other cells what to do.

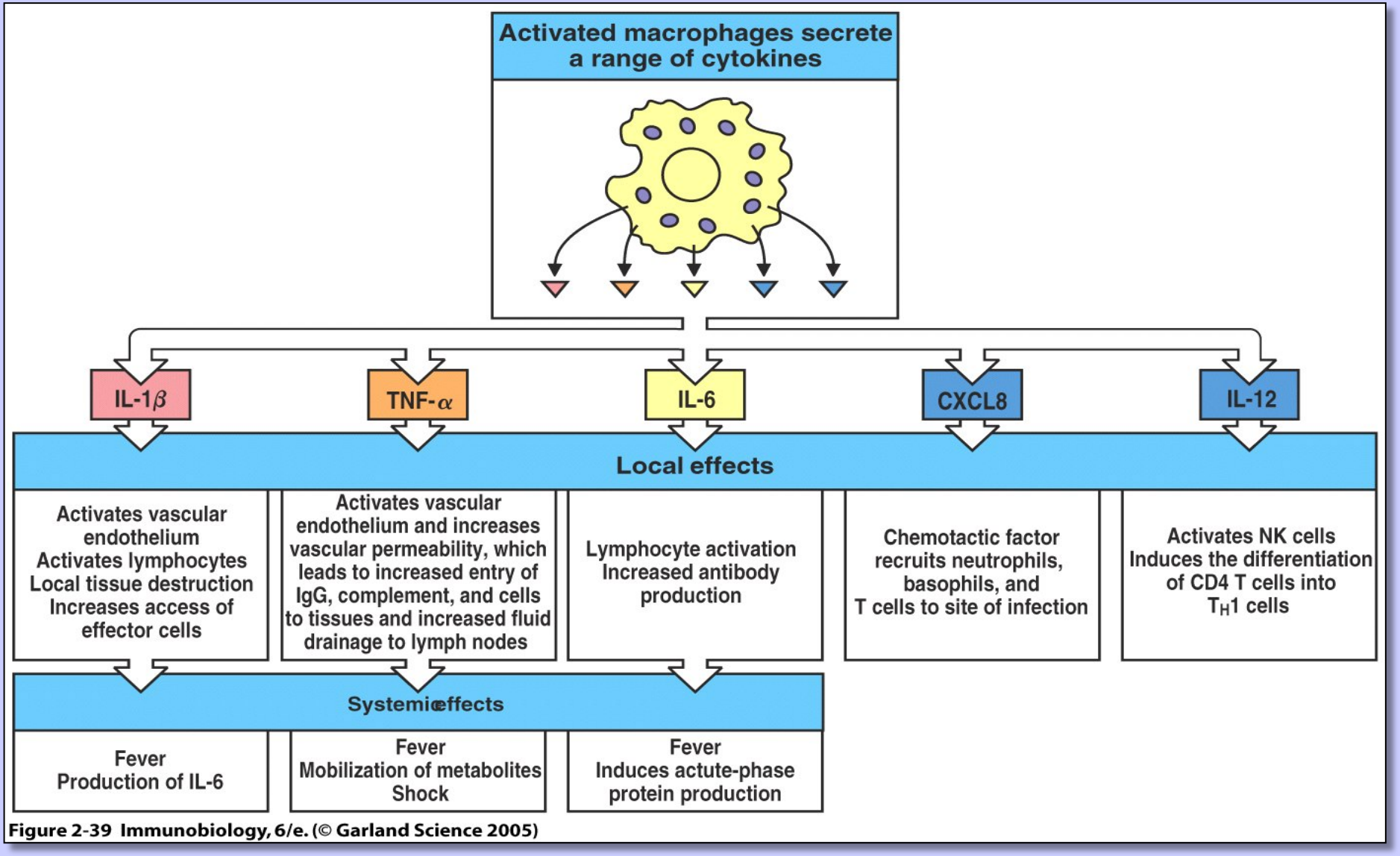
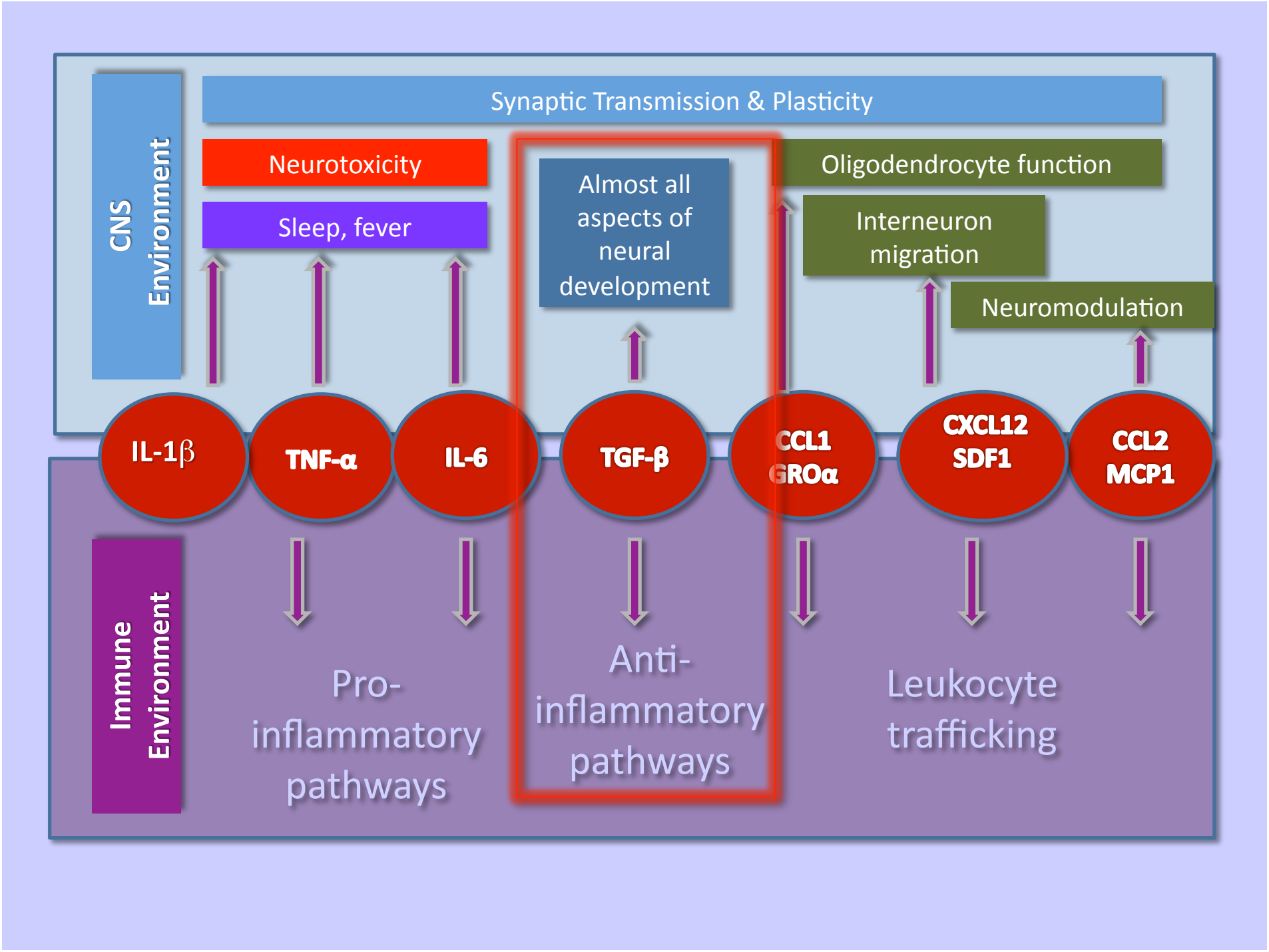


Figure 2-39 Immunobiology, 6/e. (© Garland Science 2005)


CNS Environment
Synaptic Transmission & Plasticity
Neurotoxicity
Sleep, fever
Almost all aspects of neural development
Oligodendrocyte function
Interneuron migration
Neuromodulation
IL-1β
TNF-α
IL-6
TGF-β
CCL1 GROα
CXCL12 SDF1
CCL2 MCP1
Immune Environment
Pro-inflammatory pathways
Anti-inflammatory pathways
Leukocyte trafficking

Study population at UCD

- In 2002, we began to collect samples from families as part of the Childhood Autism Risk from Genetics and the Environment (CHARGE) study.
- The CHARGE study population was sampled from three strata of children, ages 2-5 yrs:
- 1) Children with autism (currently includes over 800 families)
- 2) Typically developing children selected from the general population without autism or other developmental disabilities (currently over 500 families enrolled)
- 3) Children with developmental disabilities without autism (currently 350 families enrolled)

Do we see changes in cytokines in the plasma of children with ASD?

- Several studies have described alterations in cytokine profiles associated with autism.
- The regulatory cytokine TGF β has been linked to ASD in multiple studies.
  - Other studies describe decreased levels of TGF β in blood samples from individuals with ASD.
  - Ashwood *et al* found that lower TGF β correlated with more severe behavioral scores in ASD children.
  - Lower TGF β in peripheral blood, TGF β levels in post-mortem brain and cerebrospinal fluid samples were higher in ASD subjects than controls.

Other cytokine/chemokines altered in ASD

- MIF (macrophage inhibitory factor) a pro-inflammatory immune regulator that is constitutively expressed in brain tissues, and has important impacts on neural and endocrine systems.
  - Autism subjects with the highest levels of plasma MIF were found to have the most severe behavior.
  - Grigorenko EL, et al. Pediatrics 2008

YBRBI 1602
16 August 2010
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Brain, Behavior, and Immunity xxx (2010) xxx–xxx

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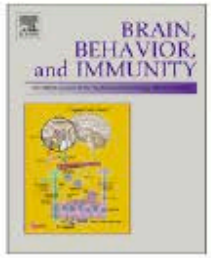


Short Communication

Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome

Paul Ashwood [a,f,*], Paula Krakowiak [b], Irva Hertz-Picciotto [b,f], Robin Hansen [c,f], Isaac Pessah [d,f], Judy Van de Water [e,f]

- Plasma levels of IL-6, IL-8, IL-1β and IL-12p40 are significantly higher in the ASD (n=97) group when compared to TD (n=87) and DD (n=39) controls.

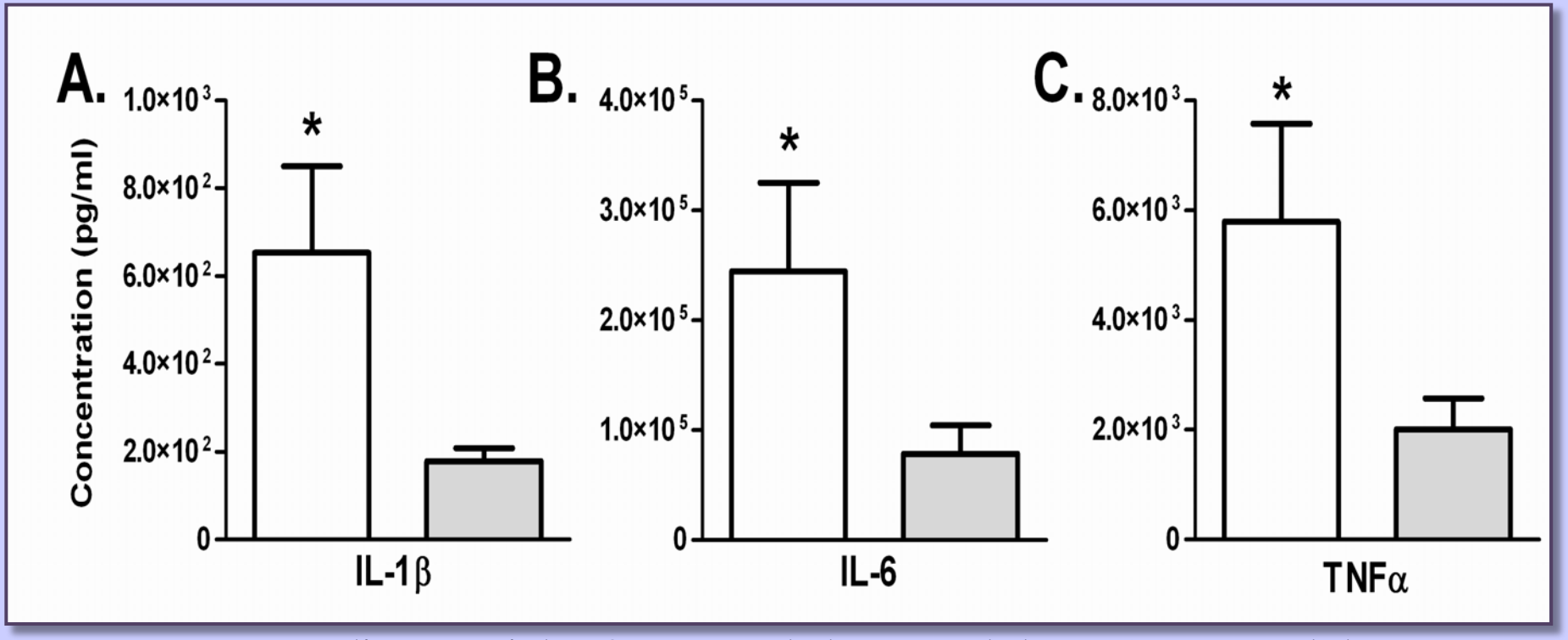
Altered T cell responses in children with autism: association with behavior

- When peripheral blood T cells were stimulated, GM-CSF, TNF α , and IL-13 were significantly increased whereas IL-12p40 was decreased in ASD relative to TD controls.
- **Increased pro-inflammatory** or TH1 cytokines were associated with **greater impairments** in core features of ASD as well as aberrant behaviors.
- In contrast, production of GM-CSF and TH2 cytokines were associated with **better cognitive and adaptive function**.

- Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Water, J.
  Brain Behav Immun. 2010 Sep 9. [Epub ahead of print]

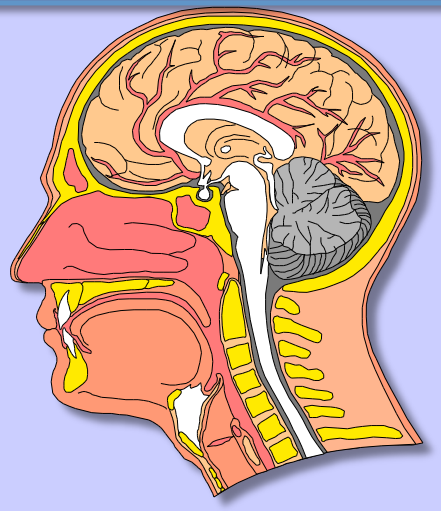
Differential monocyte responses to TLR ligands in children with autism spectrum disorders. Enstrom, et al

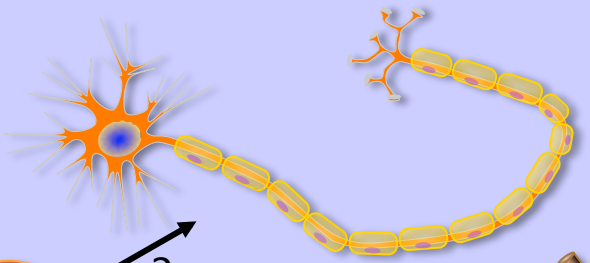
Brain Behavior and Immunity 2010; 24(1):64-71

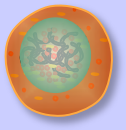


- Concentration (log-scale) of IL-1β (A), IL-6 (B), and TNFα (C) in monocyte cell culture supernatants following stimulation with TLR 2 ligands.
- Autism (white bars); typically developing controls (grey bars). * $P < 0.05$.

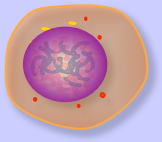
What about in the brain itself?



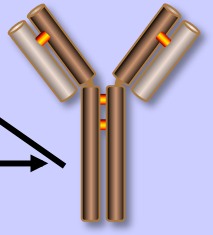




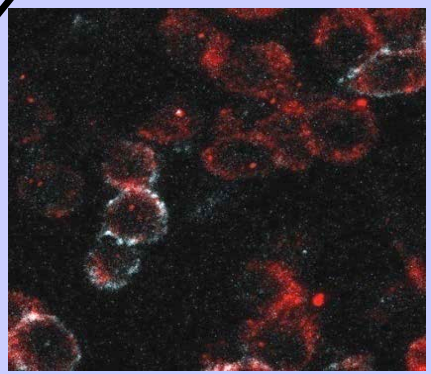
T-cell



B-cell



Immunoglobulins



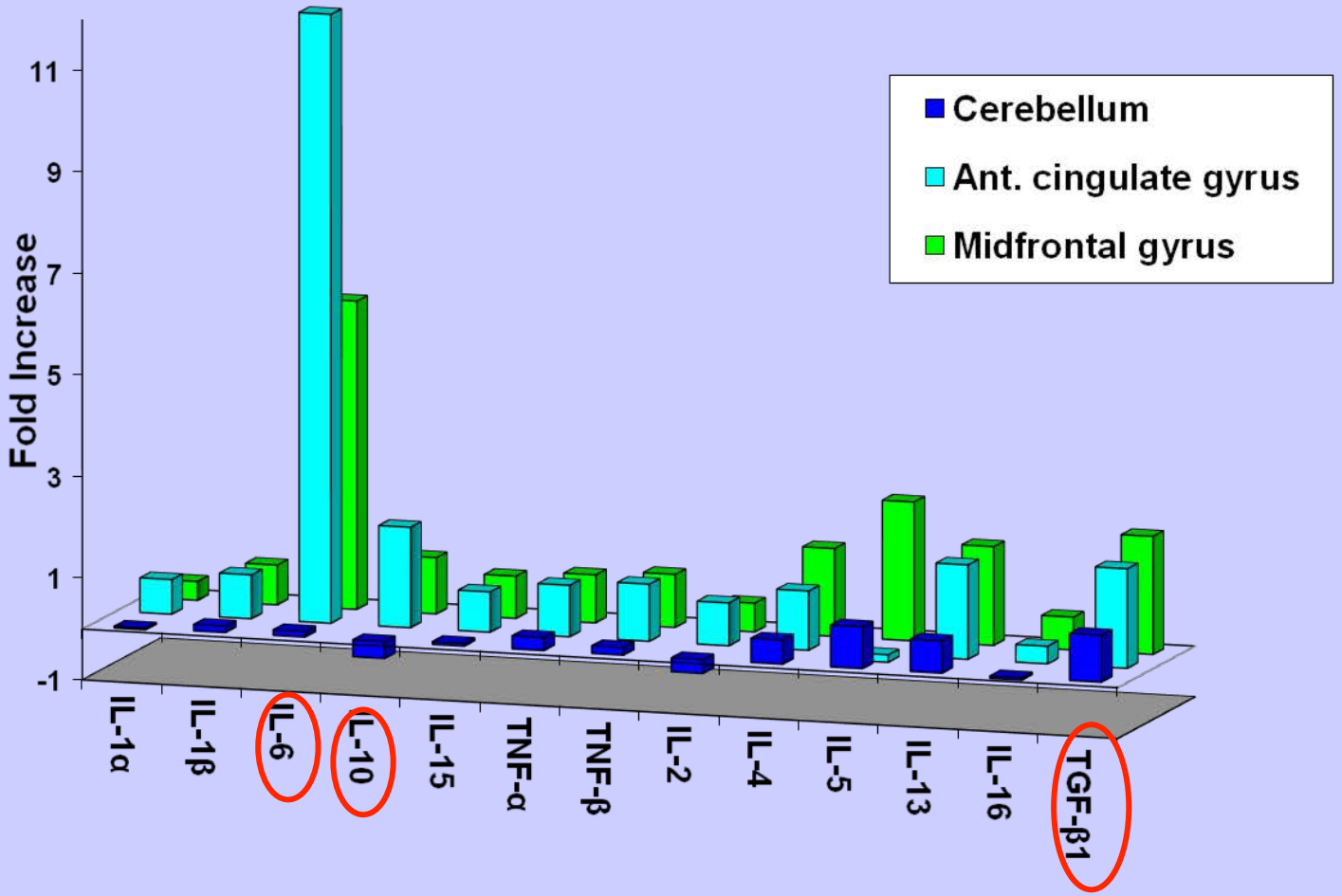
11 cases of autism

12 controls

Assessment of Adaptive or specific immune reaction: Quantification of lymphocyte infiltration, immunoglobulin deposition or complement activation

There was no evidence of any infiltration by T- or B-lymphocytes or immunoglobulin deposition in any of the brain regions studied.

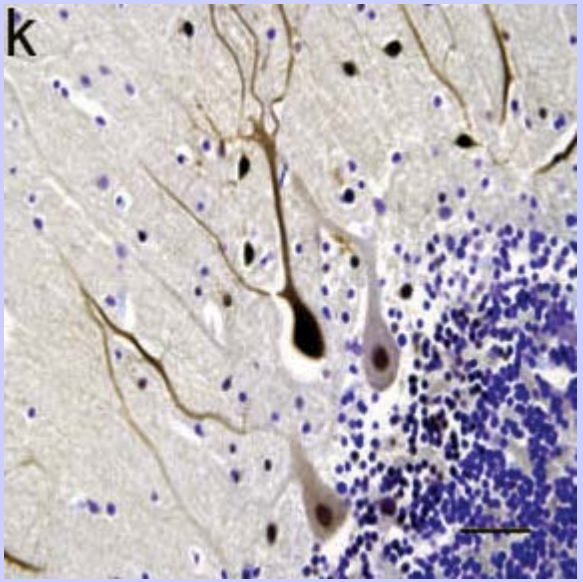
Autism: Cytokine Profile in Brain



Vargas DL et al. Ann Neurol 2005

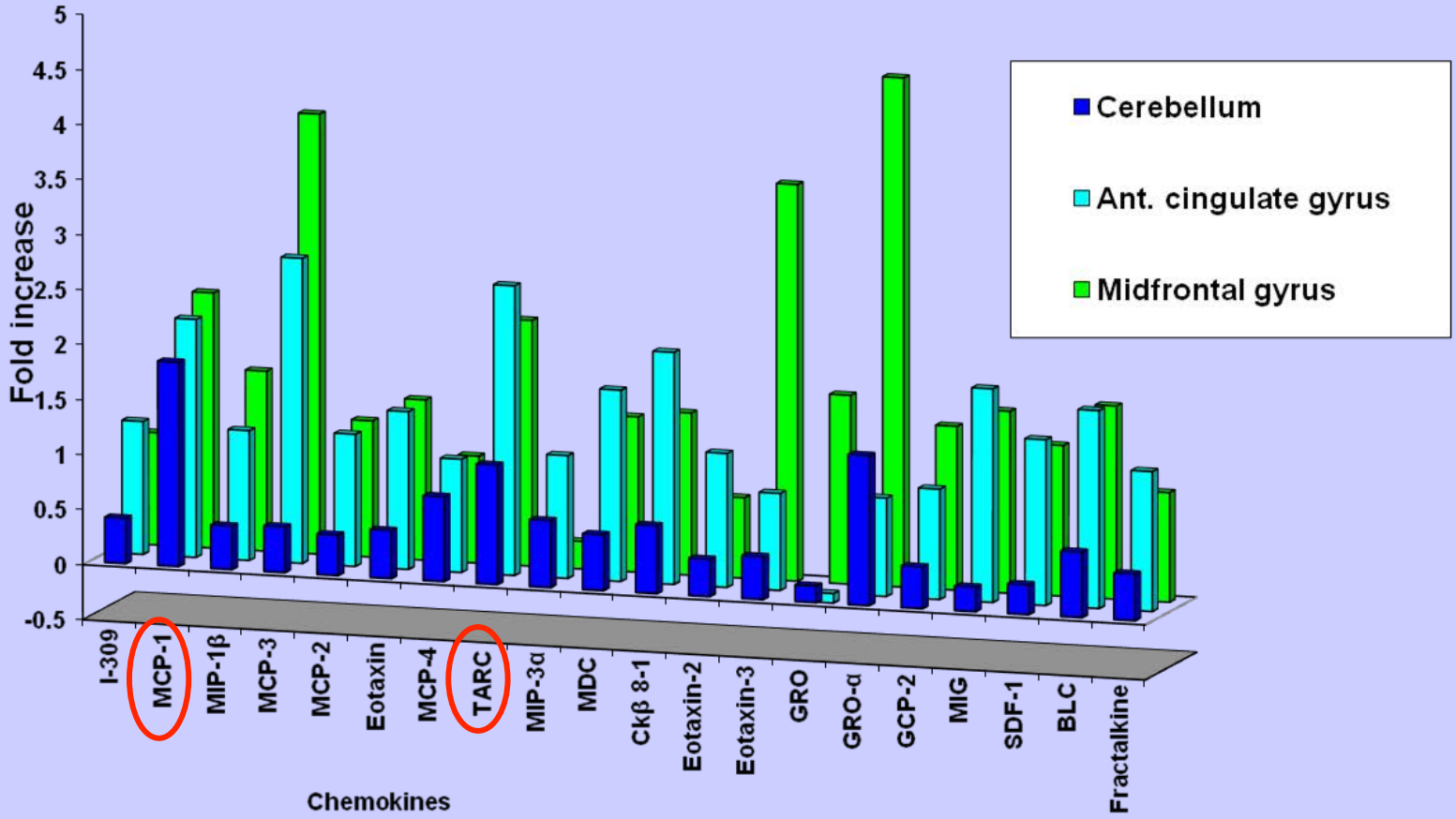
Cytokine profile in autism

- Subsets of pro-inflammatory and anti-inflammatory cytokines are increased in the brain of autistic patients.
- Cytokines are produced in the brain by neurons and neuroglial cells



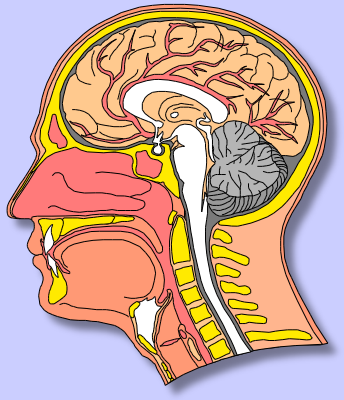
Vargas DL et al. Ann Neurol 2005

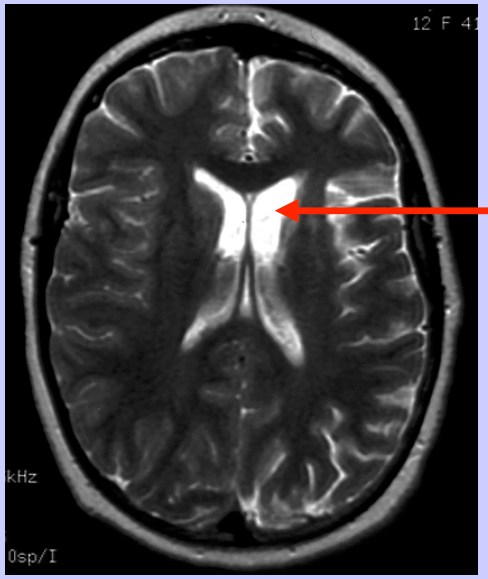
Autism: Chemokine Profile in Brain



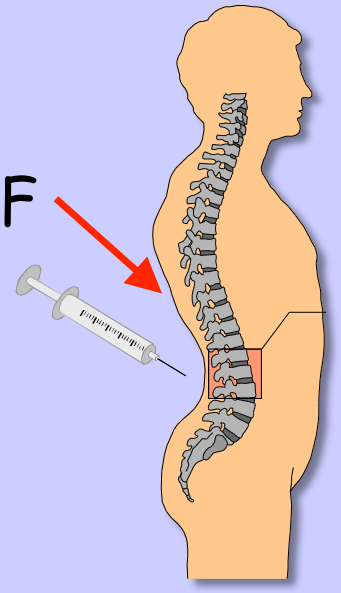
Vargas DL et al. Ann Neurol 2005

How were they able to assess inflammatory markers in-vivo?





CSF



Cerebrospinal fluid
- CSF-
- Cells
- Proteins
- Immunoglobulins
- Oligoclonal bands

▼

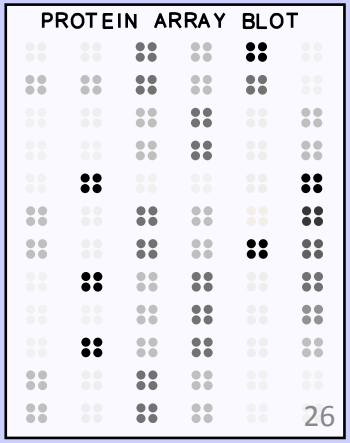
Cytokines
Chemokines

Assessment of cytokines and chemokines in CSF: Expression profile and quantification

→

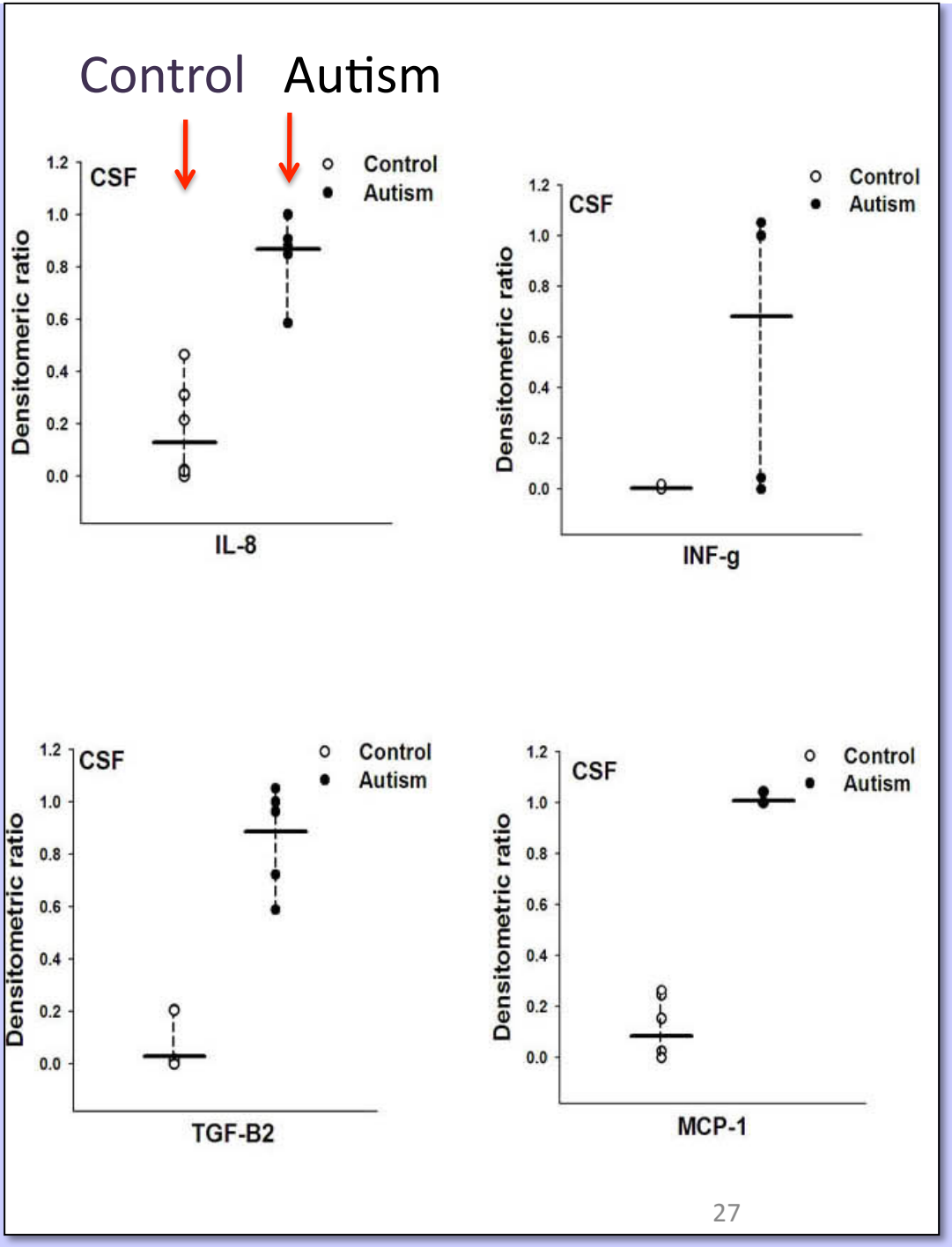
7 autism cases
7 control cases

→



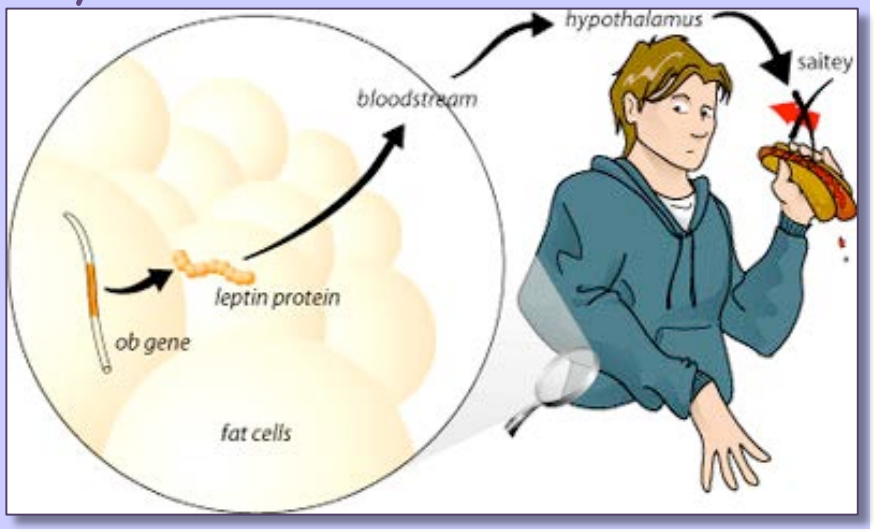
Autism:
In-vivo markers of inflammation in the cerebrospinal fluid were found.

Vargas DL et al. Ann Neurol 2005



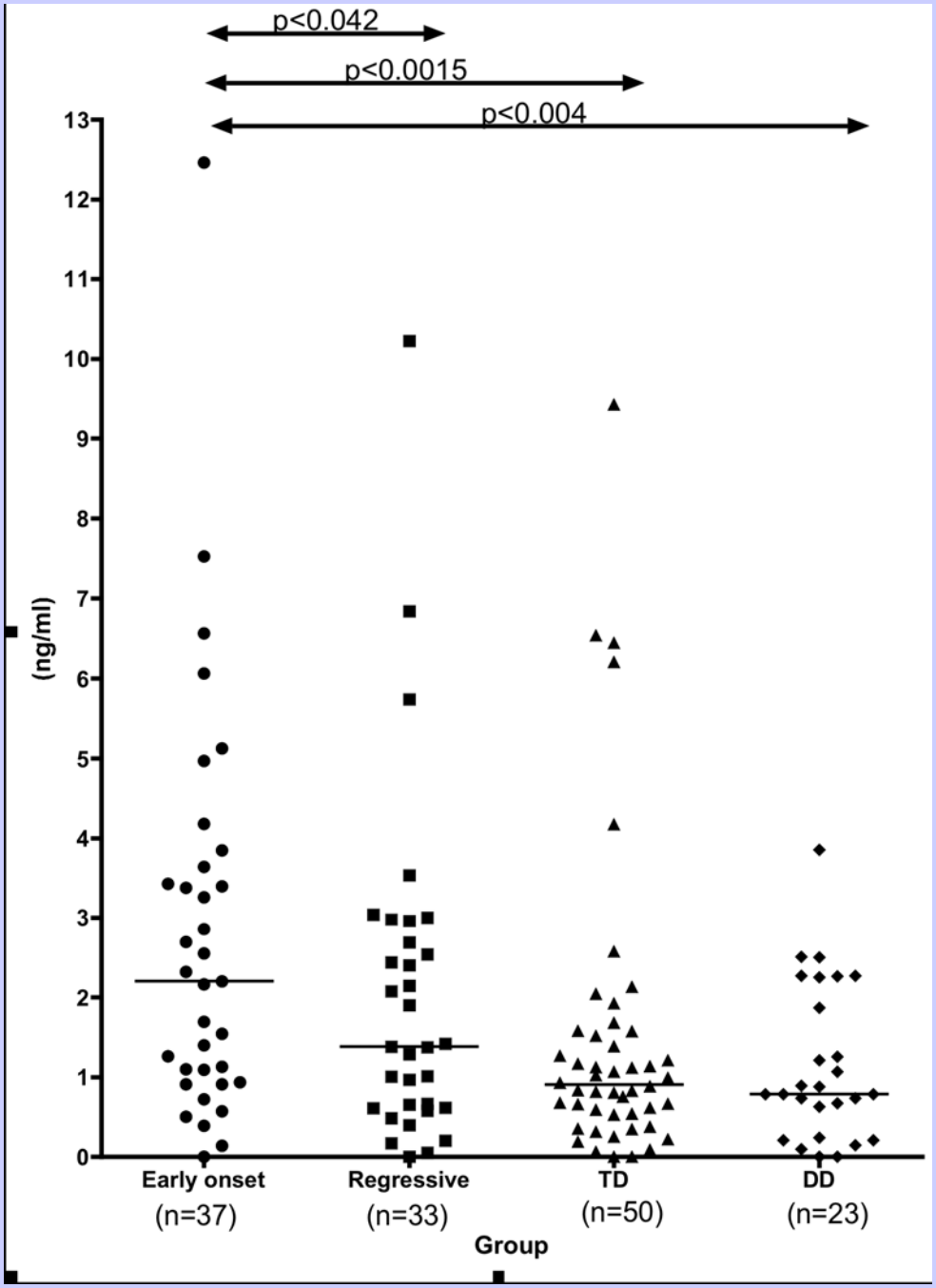
Leptin, a newly discovered cytokine

- Adipokines such as leptin are an examples of molecules that interface between immune and metabolic regulation.
- Leptin signals the brain that sufficient food is stored as fat.
- In the following study, we compared plasma leptin levels in a population of well-defined children with autism and age-matched controls, with and without developmental disabilities.



Plasma Leptin Levels are Elevated in Autism: Association with an Early Onset Phenotype? Ashwood et al, JADD, 2008

- The cytokine leptin was analyzed in children with early onset vs regressive autism
- Can begin to separate phenotypes based on biologic outcomes



What does this mean?

- Leptin controls intracellular metabolism.
- Decreased leptin leads to increased infections.
- Increased leptin, see increased frequency of autoimmunity.

What might be the outcome of immune dysregulation such as elevated inflammatory cytokines?

- One potential outcome is the increased susceptibility towards the generation of autoantibodies.
- We have examined plasma from children with an ASD for autoantibodies to brain proteins using:
  - Immunohistochemistry
  - Western blot analysis

Brain, Behavior, and Immunity 23 (2009) 64–74

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

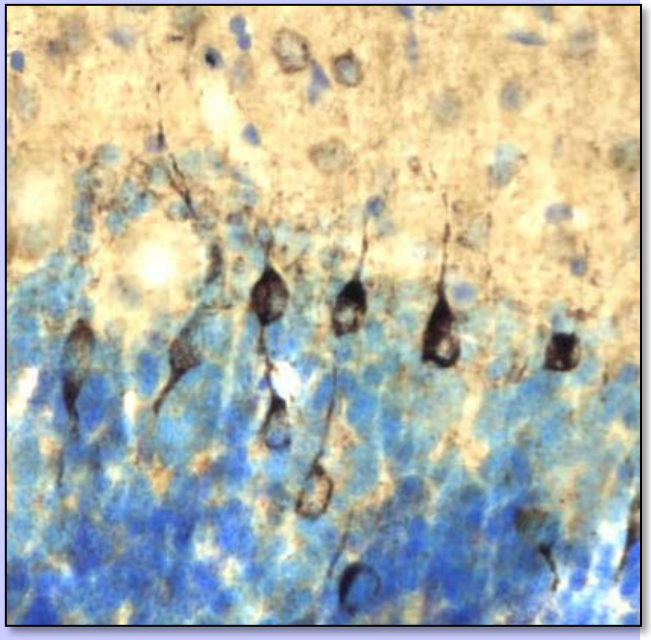
journal homepage: www.elsevier.com/locate/ybrbi

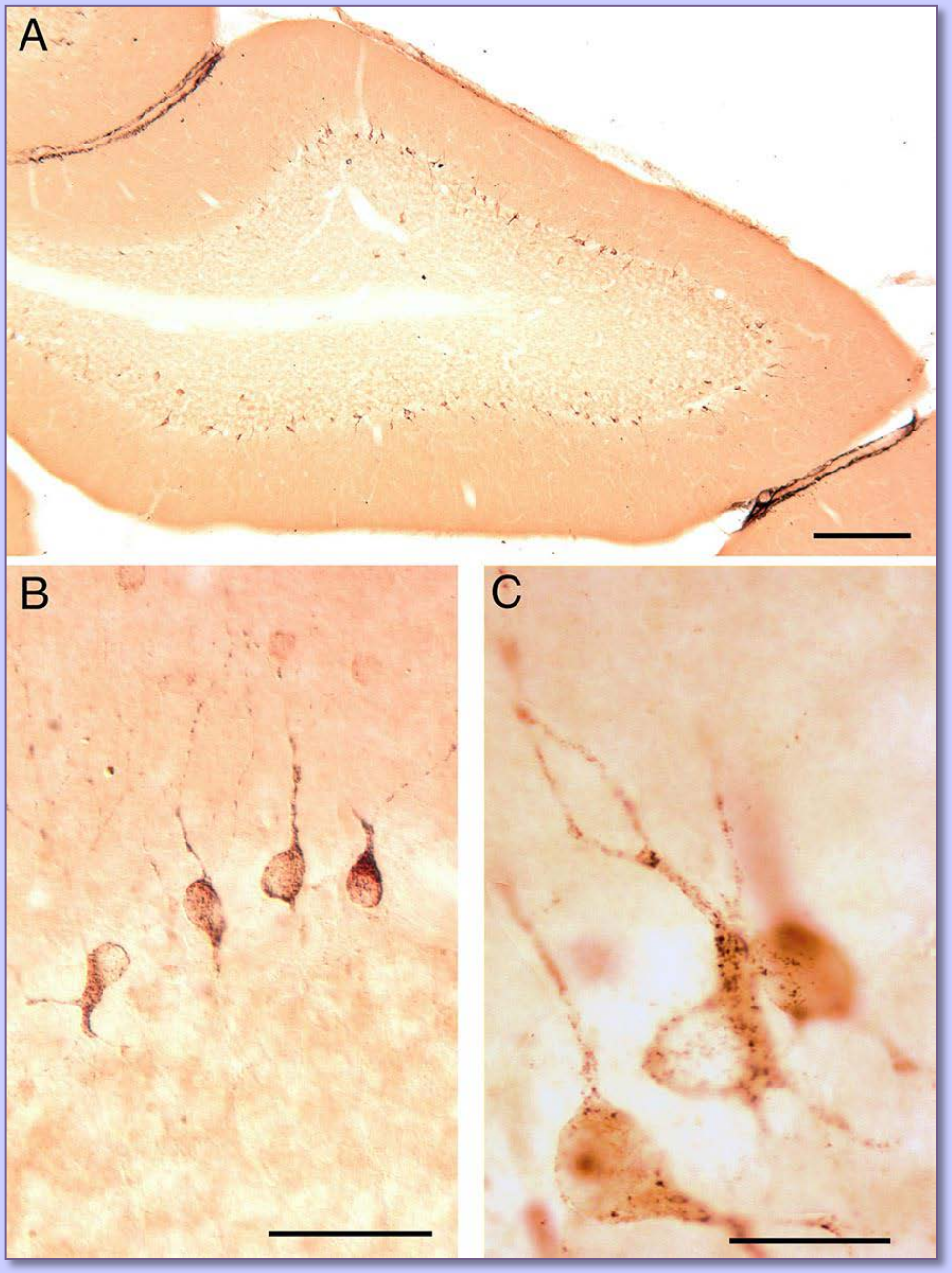
Detection of autoantibodies to neural cells of the cerebellum in the plasma of subjects with autism spectrum disorders

Sharifia Wills [a,e], Maricel Cabanlit [a,e], Jeff Bennett [b,d], Paul Ashwood [c,d,e], David G. Amaral [b,d], Judy Van de Water [a,d,e,*]

Autoantibodies from children with ASD-

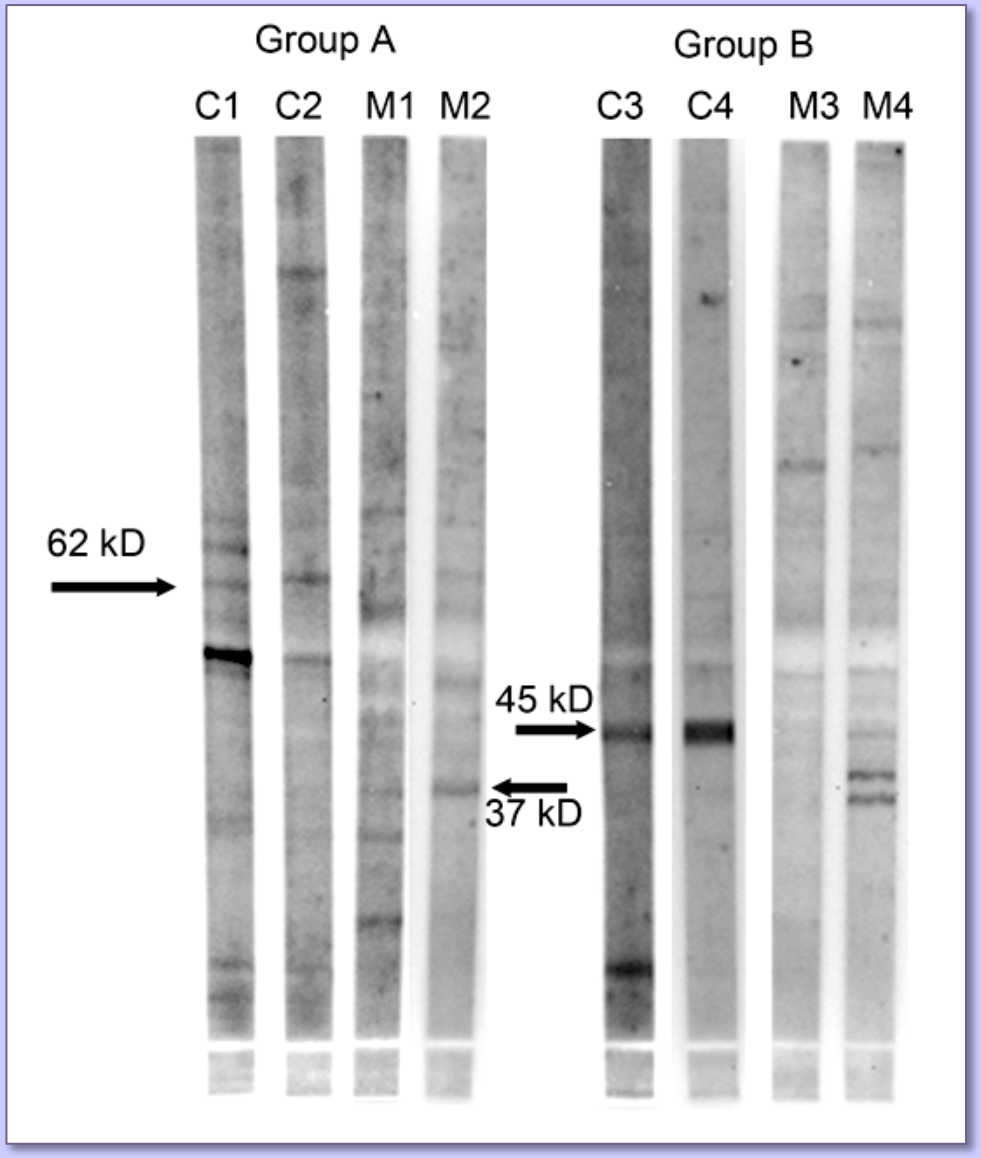
Immunohistochemical and Western blot analysis of autoantibody localization in cerebellum of Rhesus monkeys





- The golgi cell of the cerebellum was strongly reactive when probed with antibodies from children with autism.
- Intense golgi cell staining was observed in ~21% of patients with ASD compared with 0% of normal controls.

Western blot of monkey cerebellum



- Blot was run with plasma from children with autism and the presence of bands determined

Goines, et al,
Submitted BBI, 2010

Western blot analysis of monkey cerebellum

	Child IgG Targets in Cerebellum				*P* values			
	ASD n=70	**AU n=207**	**AU +ASD n=277**	**TD n=189**	**ASD** vs. **TD**	**AU** vs. **TD**	**AU +ASD** vs. **TD**	**AU** vs. **ASD**
45	5 (7.1%)	20 (9.7%)	25 (9%)	7 (3.6%)	NS	0.017	0.025	NS
62	12 (16%)	17 (8.2%)	29 (10%)	16 (8.2%)	0.043	NS	NS	0.043

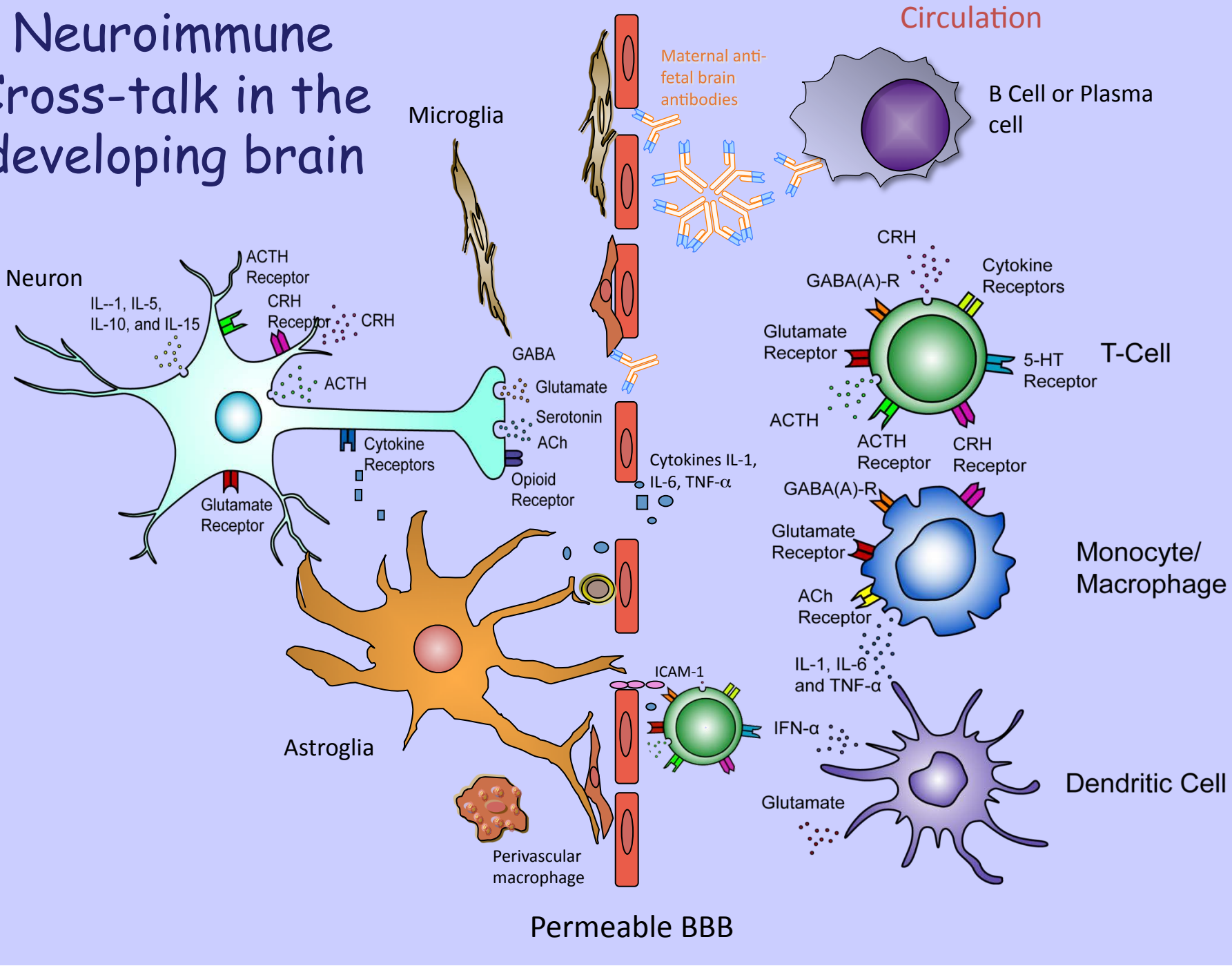
Significant behavioral associations for children with and without IgG reactivity towards the 45 kDa protein.

	ASD			AU			TD			All Children		
Test	**Mean Score (SD)**		***P***	**Mean Score (SD)**		***P***	**Mean Score (SD)**		***P***	**Mean Score (SD)**		***P***
	45+	**45-**		**45+**	**45-**		**45+**	**45-**		**45+**	**45-**	
ABC 2: Lethargy	8.2 (7)	9 (7.9)	ns	12.9 (9.6)	12.3 (7.1)	ns	0.5 (1.22)	0.47 (1.6)	ns	9.3 (9.3)	6.7 (7.9)	0.05
ABC 3: Stereotypy	3.2 (2.8)	3.3 (3.5)	ns	7.8 (6.4)	5.7 (4.2)	ns	0 (0)	0.1 (0.4)	ns	5.2 (6)	2.9 (4)	0.01
MSEL	69 (26.1)	68.4 (17.8)	ns	53.5 (6.4)	59.5 (17.2)	ns	108 (13.8)	105.1 (17.7)	ns	67.1 (24.9)	80.6 (27.8)	0.005
VABS	68.5 (19.6)	70.2 (12.7)	ns	58.6 (7)	62.9 (12)	ns	100.8 (17.7)	104.9 (14.9)	ns	68.7 (20.4)	82 (24)	0.0008

Significant behavioral associations for children with and without IgG reactivity towards the 62 kDa protein.

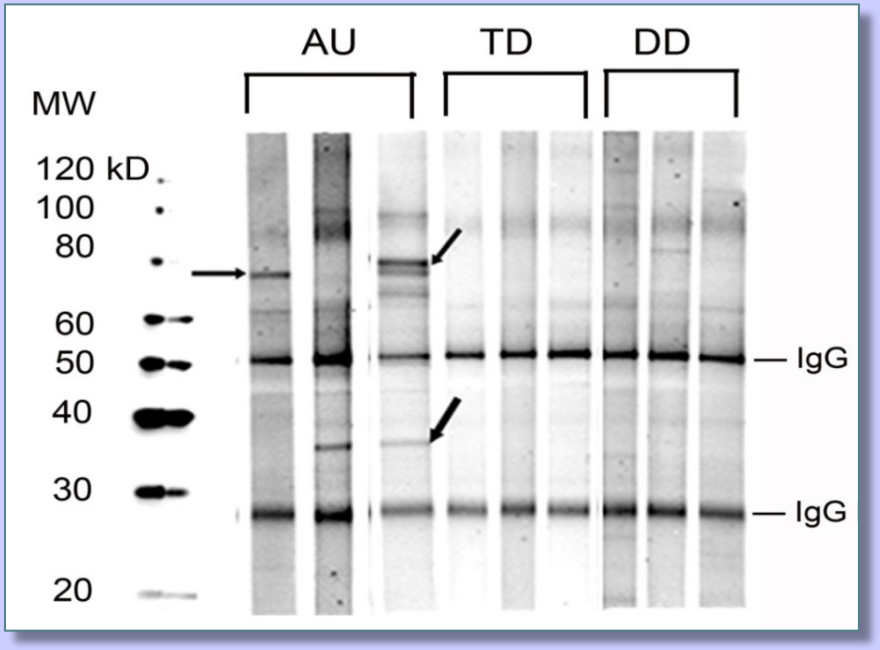
Test	ASD		P	AU		P	TD		P	All Children		P
	Mean Score (SD)			Mean Score (SD)			Mean Score (SD)			Mean Score (SD)		
	62+	62-		62+	62-		62+	62-		62+	62-	
Total ABC	68.1 (40.9)	38.7 (25.)	0.02	48.3 (25.6)	53.1 (26.1)	ns	10.9 (14.1)	7.1 (10)	ns	38.9 (35.1)	30.1 (29.5)	ns
ABC 2: Lethargy	16.6 (11.9)	7.5 (5.9)	0.01	10.3 (7.5)	12.6 (7.3)	ns	0.4 (0.8)	0.5 (1.7)	ns	8.4 (9.8)	6.7 (7.8)	ns
ABC 3: Stereotypy	5.6 (3.5)	2.9 (3.3)	0.01	5.2 (4)	6 (4.6)	ns	0.07 (0.3)	0.09 (0.4)	ns	3.5 (3.9)	3 (4.2)	ns
ABC 5: Inappropriate Speech	3.5 (3.4)	2.8 (2.8)	ns	3 (2.9)	2.8 (2.8)	ns	0.9 (1.5)	0.4 (0.9)	ns	2.4 (2.9)	1.8 (2.5)	0.04
VABS	67.7 (12.3)	70.5 (13.5)	ns	65.1 (15.8)	62.1 (11.1)	ns	97.5 (15.4)	105.5 (14.8)	0.02	76.9 (20.9)	81.5 (24.3)	ns

Neuroimmune Cross-talk in the developing brain



Results of First Study

Study population	Number 37 kD &73 kD band positive (%)
AU (n=61)	7 (12%)
TD (n=62)	0 (0%)
DD (n=40)	0 (0%)



Neurotoxicology 29:226-231, 2008 E-Pub ahead of print (November 2007)

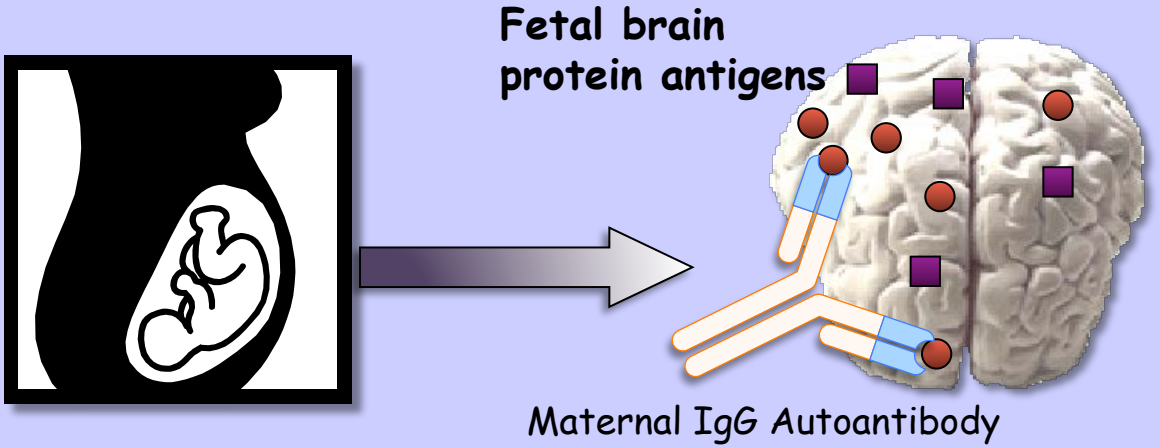
Abbreviations: AU = Autism; TD = Typically Developing; DD = Developmental delays

Expanded Study: Specificity Remains

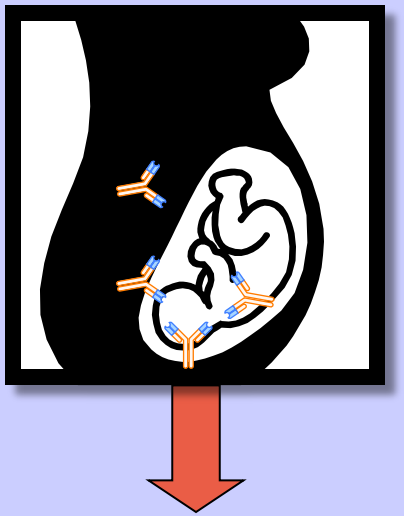
Study Population (n)	37&73kD*	39&73kD	37&73kD or 39&73kD	37 & 39 & 73kD
AU (n=204)	**12 (6%)**	10 (5%)	**22 (11%)**	**6 (3%)**
ASD (n=71)	3 (4%)	9 (13%)	12 (17%)	0 (0%)
AU and ASD (n=275)	15 (5%)	19 (7%)	34 (12%)	6 (2%)
TD (n=183)	**0 (0%)**	3 (2%)	3 (2%)	**0 (0%)**
Significance (p-value)	37&73kD	39&73kD	37&73kD or 39&73kD	37 & 39 & 73kD
AU vs TD	0.0005	0.093	0.0003	0.0314
ASD vs TD	0.0212	0.0007	<0.0001	NA
AU & ASD vs TD	0.0006	0.0124	<0.0001	0.0856
AU vs ASD	0.766	0.0525	0.209	0.344

Abbreviations: AU = Autism; TD = Typically Developing; DD = Developmental delays. Submitted: J. Autoimmunity

The Maternal Autoantibody Model

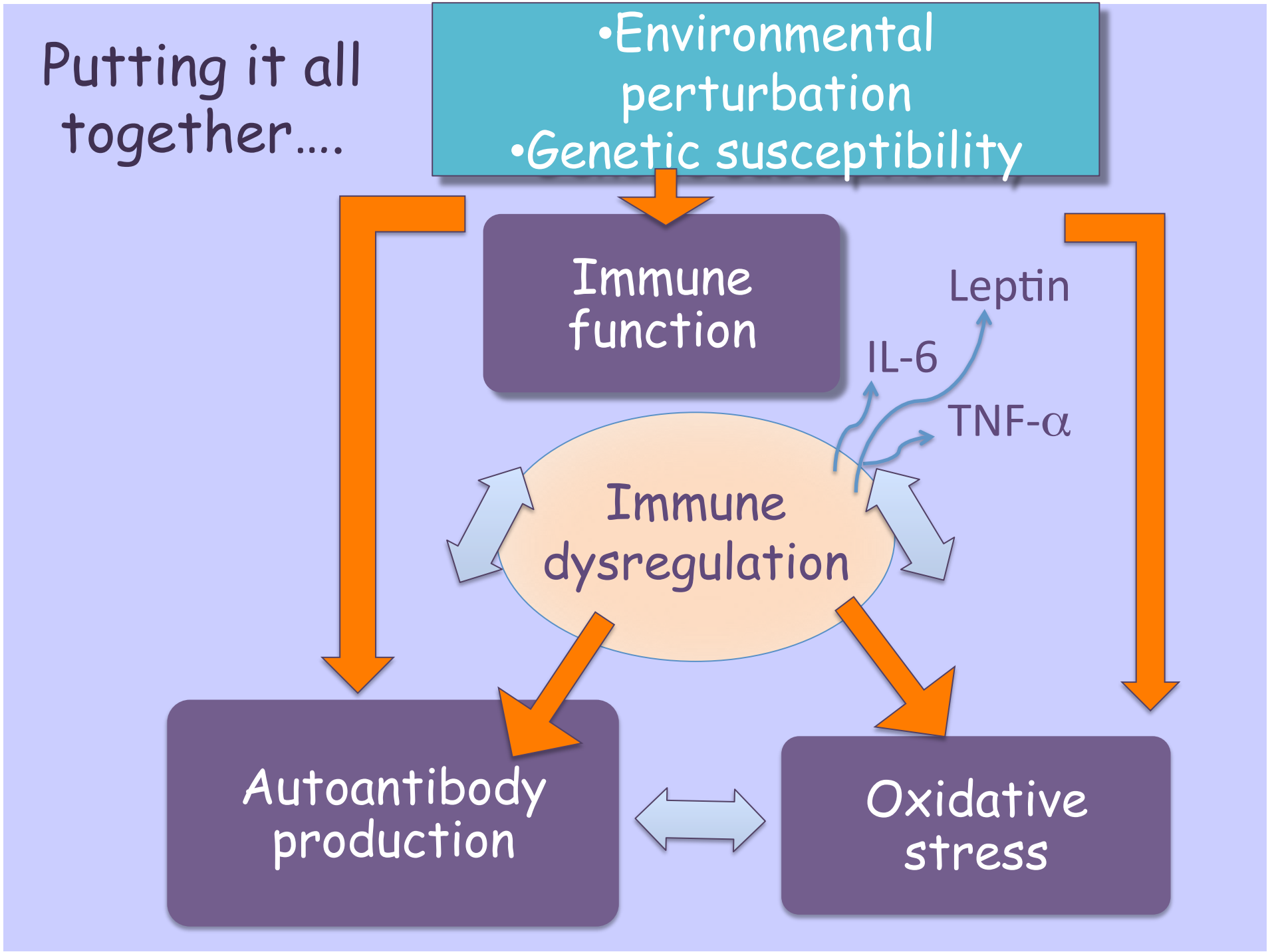


Autoantibodies can cross the placenta and bind to proteins present in the developing fetal brain.



Altered Neurodevelopment

Putting it all together….



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