Rutgers Brain Health Institute

2015-2016

Plenary Seminar Series

No Quiet Surrender: Molecular Guardians In MS Brain

Lawrence Steinman Stanford University

November 12, 2015

Disclosures

- Steinman consults for Receptos (now Celgene), AbbVie, Teva, EMD Serono, Novartis Atreca, Raptor
- Steinman has received research grants from Pfizer, Biogen
- Steinman holds stock or options in Raptor, Tolerion, Atreca, Transparency Life Sciences

No quiet surrender: molecular guardians in multiple sclerosis brain

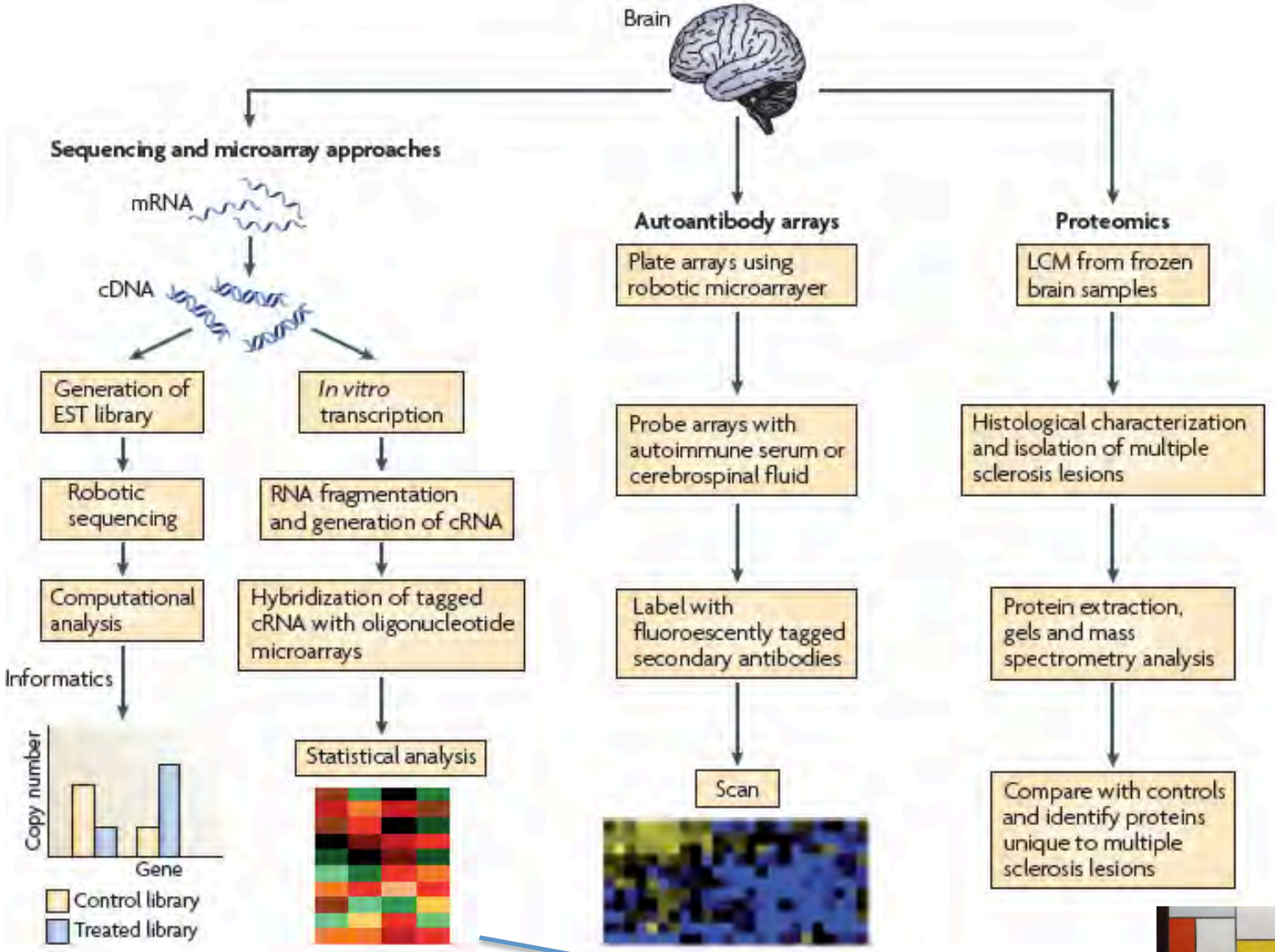
Lawrence Steinman

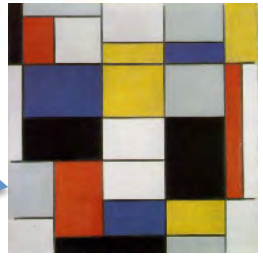
Department of Neurology and Neurological Sciences, Stanford University, Stanford, California, USA.

The brain under immunological attack does not surrender quietly. Investigation of brain lesions in multiple sclerosis (MS) reveals a coordinated molecular response involving various proteins and small molecules ranging from heat shock proteins to small lipids, neurotransmitters, and even gases, which provide protection and foster repair. Reduction of inflammation serves as a necessary prerequisite for effective recovery and regeneration. Remarkably, many lesion-resident molecules activate pathways leading to both suppression of inflammation and promotion of repair mechanisms. These guardian molecules and their corresponding physiologic pathways could potentially be exploited to silence inflammation and repair the injured and degenerating brain and spinal cord in both relapsing-remitting and progressive forms of MS and may be beneficial in other neurologic and psychiatric conditions.

In the next 50 minutes I shall share with you some “tractable” targets derived from various “omics”, without the aid of “genomics”. I shall describe the following:

1. The inhibitory neurotransmitter GABA is immune suppressive
2. Angiotensin Receptors are in MS Lesions
   ACE inhibition is beneficial in animal models
3. Immune suppressive lipids in the myelin sheath
4. Infamous amyloid proteins provide protection, not harm in neuroinflammatory conditions
5. PPARs are targetable natural “brakes” on neuroinflammation. They may also help explain gender disparity in MS





Mondrian, Composition A, 1920

Was this a cluster analysis from microarrays?

doi:10.1038/nature06559 nature

ARTICLES

Proteomic analysis of active multiple sclerosis lesions reveals therapeutic targets

May H. Han[1]*, Sun-Il Hwang[3]*, Dolly B. Roy[4]*, Deborah H. Lundgren[3], Jordan V. Price[1], Shalina S. Ousman[1], Guy Haskin Fernald[5], Bruce Gerlitz[6], William H. Robinson[2], Sergio E. Baranzini[5], Brian W. Grinnell[6], Cedric S. Raine[7], Raymond A. Sobel[8], David K. Han[3] & Lawrence Steinman[1]

REPORTS

The Influence of the Proinflammatory Cytokine, Osteopontin, on Autoimmune Demyelinating Disease

Dorothée Chabas,[1]* Sergio E. Baranzini,[2]* Dennis Mitchell,[1] Claude C. A. Bernard,[3] Susan R. Rittling,[4] David T. Denhardt,[4] Raymond A. Sobel,[5] Christopher Lock,[1] Marcela Karpuj,[1,2] Rosetta Pedotti,[1] Renu Heller,[6]† Jorge R. Oksenberg,[2]† Lawrence Steinman[1]†‡

In the next 50 minutes I shall share with you some “tractable” targets derived from various “omics”, without the aid of “genomics”. I shall describe the following:

1. **The inhibitory neurotransmitter GABA is immune suppressive**

Proteomic and Microarray Studies on Tissues Point to the GABA Pathway

Table 1. GABA pathway machinery changes in MS

GABA gene	Description	Up/down in tissue type	Reference	Type
GAD	Glutamic acid decarboxylase GABA synthetic enzyme	Down 4/4 MS brains	Lock et al	Microarray
		Unique to Acute Plaque	Han et al	Proteomics
		Down Motor cortex	Dutta et al	Microarray
GAT-1	GABA reuptake transporter	Unique to Acute Plaque	Han et al	Proteomics
GABA-A-R α1	GABA A receptor α1 subunit	Down 3/4 MS brains	Lock et al	Microarray
GABA-A-R β3	GABA A receptor β3 subunit	Down Motor cortex	Dutta et al	Microarray
GABA-A-R ε	GABA A receptor ε subunit	Up 3/4 MS brains	Lock et al	Microarray
		Down Motor cortex	Dutta et al	Microarray
		Down Chronic Active Plaque	Tajouri et al	Microarray
GABA-A-R γ2	GABA A receptor γ2 subunit	Down 3/4 MS brains	Lock et al	Microarray
		Down Motor cortex	Dutta et al	Microarray
		Down Chronic Active Plaque	Tajouri et al	Microarray
		Up Acute Plaque	Tajouri et al	Microarray
GABRAP	GABA receptor associated protein	Unique to Chronic Plaque	Han et al	Proteomics
		Down Motor cortex	Dutta et al	Microarray

Roopa Bhat
With Alfred N.,
Stockholm 2009

PNAS PNAS

Inhibitory role for GABA in autoimmune inflammation

Roopa Bhat[a,1], Robert Axtell[a], Ananya Mitra[b], Melissa Miranda[a], Christopher Lock[a], Richard W. Tsien[b], and Lawrence Steinman[a]

[a]Department of Neurology and Neurological Sciences and [b]Department of Molecular and Cellular Physiology, Beckman Center for Molecular Medicine, Stanford University, Stanford, CA 94305

Contributed by Richard W. Tsien, December 31, 2009 (sent for review November 30, 2009)

GABA, the principal inhibitory neurotransmitter in the adult brain, has a parallel inhibitory role in the immune system. We demonstrate that immune cells synthesize GABA and have the machinery for GABA catabolism. Antigen-presenting cells (APCs) express functional GABA receptors and respond electrophysiologically to GABA. Thus, the immune system harbors all of the necessary constituents for GABA signaling, and GABA itself may function as a paracrine or autocrine factor. These observations led us to ask further whether manipulation of the GABA pathway influences an animal model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE). Increasing GABAergic activity ameliorates ongoing paralysis in EAE via inhibition of inflammation. GABAergic agents act directly on APCs, decreasing MAPK signals and diminishing subsequent adaptive inflammatory responses to myelin proteins.

serum (13). Because actions of exogenous GABA on inflammation and of endogenous GABA on phasic synaptic inhibition both occur at millimolar concentrations (5, 8, 9), we hypothesized that local mechanisms may also operate in the peripheral immune system to enhance GABA levels near the inflammatory focus. We first asked whether immune cells have synthetic machinery to produce GABA by Western blotting for GAD, the principal synthetic enzyme. We found significant amounts of a 65-kDa subtype of GAD (GAD-65) in dendritic cells (DCs) and lower levels in macrophages (Fig. 1*A*). GAD-65 increased when these cells were stimulated (Fig. 1*A*, DR vs. DS, and MR vs. MS). Assays of GABA in conditioned media from purified cultures of DCs, macrophages, and T cells indicated GABA secretion by these cell types (Fig. 1*B*). In contrast to the alteration in GAD-65 with stimulation, the amount of GABA collected in the conditioned media did not

GABA Metabolic Pathways

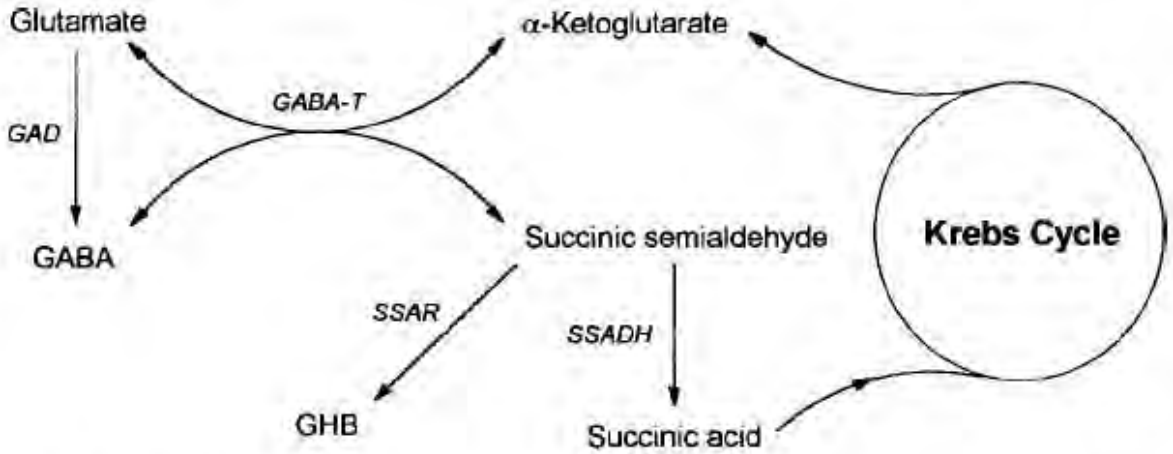
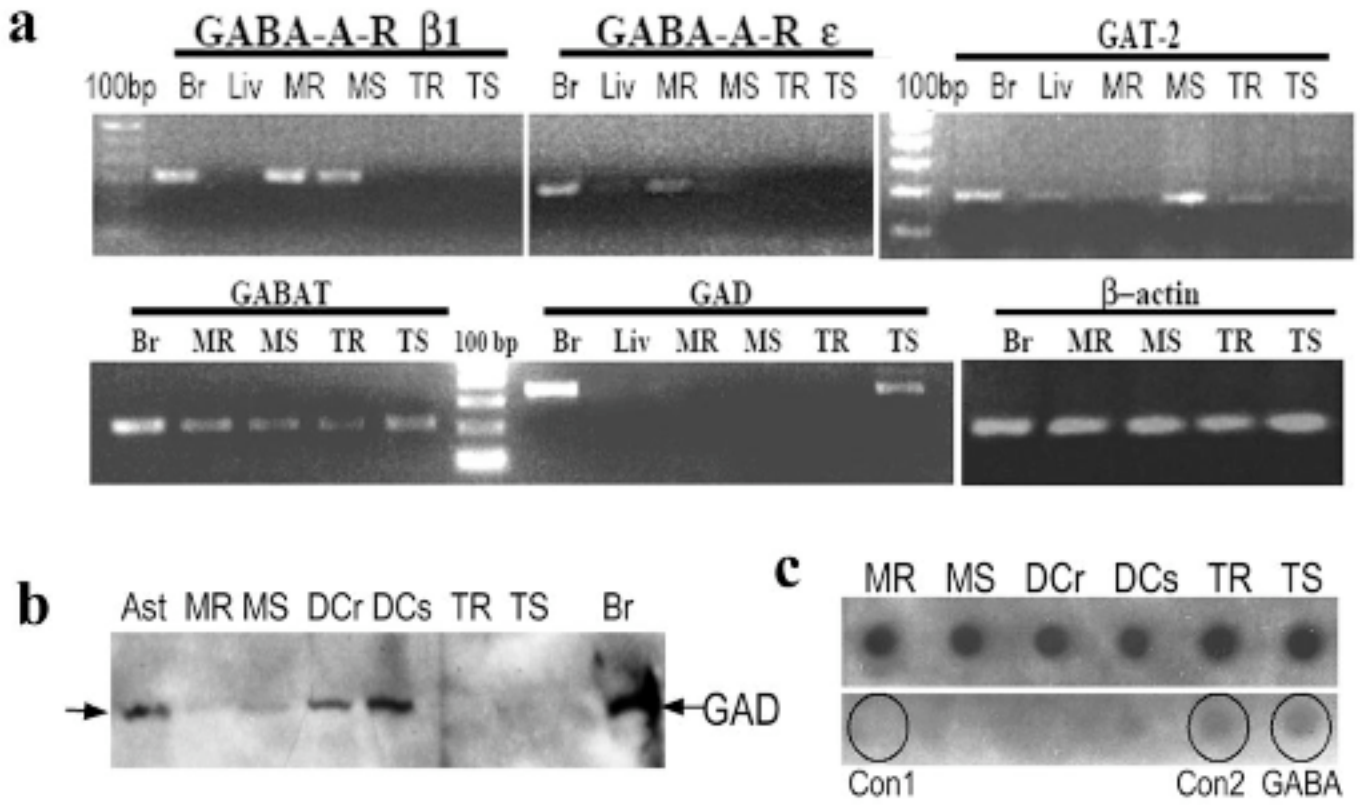


Fig. 1. GABA metabolism pathway. GABA-T: GABA transaminase; GAD: glutamic acid decarboxylase; GHB: γ-hydroxybutyric acid; SSADH: succinic semialdehyde dehydrogenase; SSAR: succinic semialdehyde reductase.

All GABA Biochemical Elements Are Present in the Immune System:

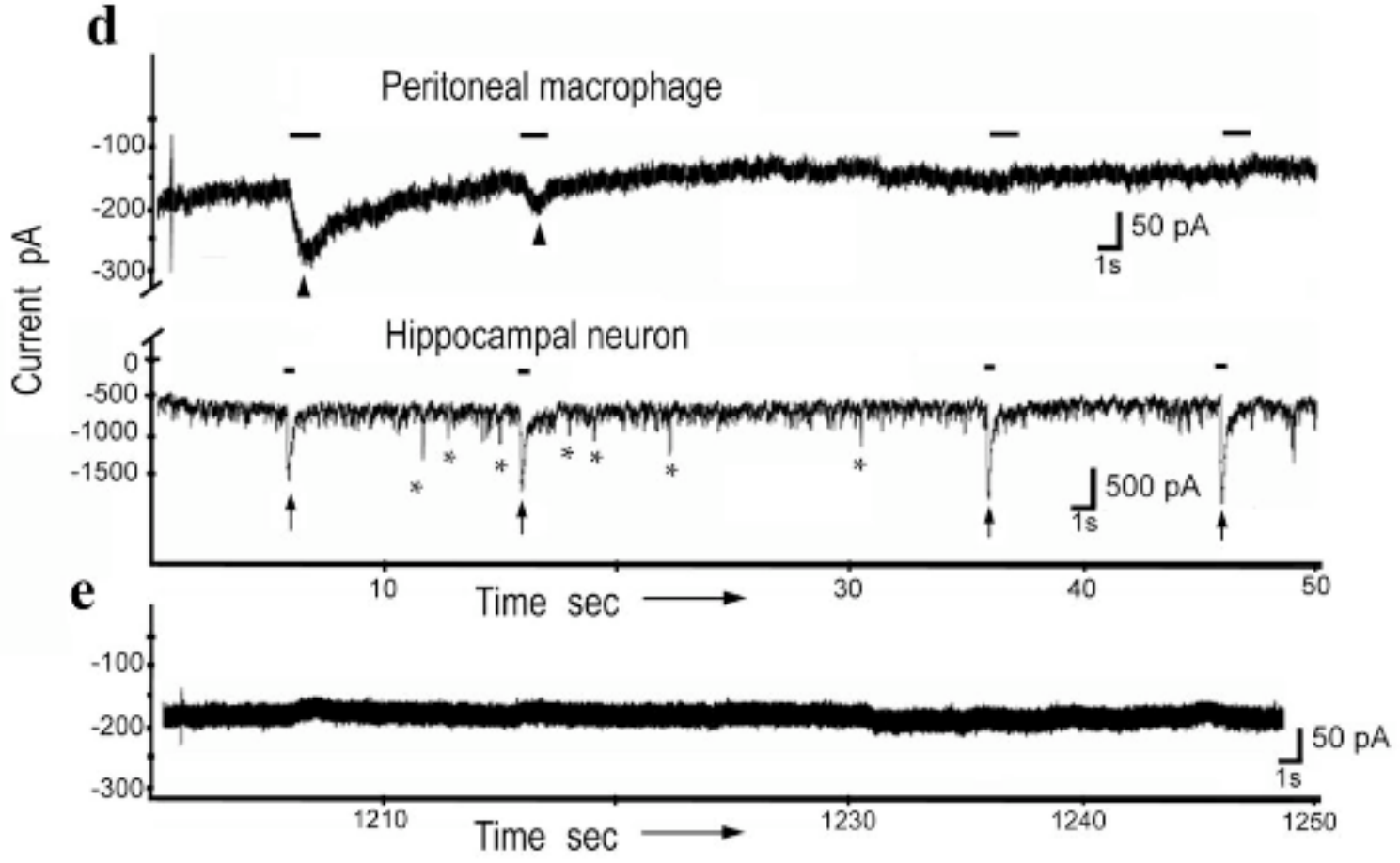
1) Synthetic Enzyme GAD
2) GABA-A-R,
3) Degradative Enzyme GABA-T
4) GABA Transporter, GAT-2



Functional GABA-R In Patch Clamped Macrophages

GABA Currents Smaller with Slower Kinetics Than Neurons

Probably Endocytosis of GABA Receptors

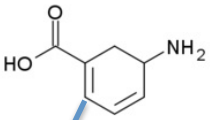


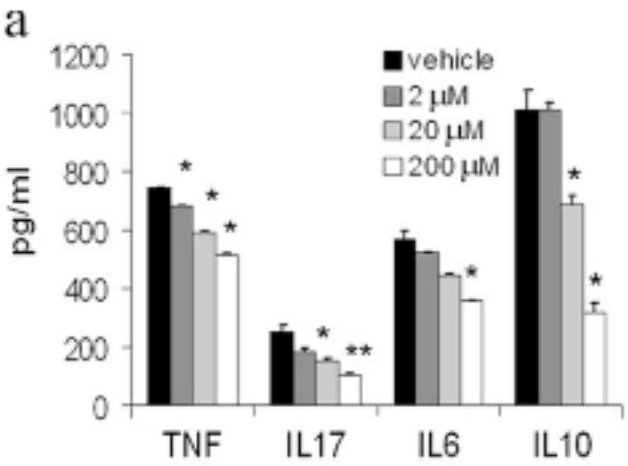
In Collaboration with Dr. Ananya Mitra

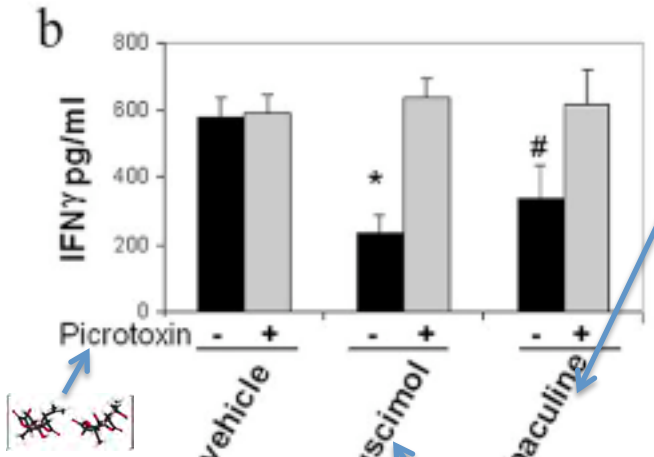
GABAergic Agents Suppress Pro-inflammatory Gamma IFN production from T cells via action On Macrophages, aka APC, via GABA-A Receptor

- Muscimol is GABA-A-R agonist
- Gabaculine is GABA-T inhibitor
- Splenocytes with APC + TCR from MOG TCR, 2D2

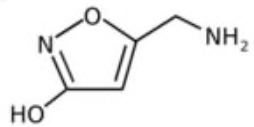
Gabaculine is is a naturally occurring neurotoxin first isolated from the bacteria *Streptomyces toyacaensis,[1] which acts as a potent irreversible GABA transaminase inhibitor,[2][3] and also a GABA reuptake inhibitor.*



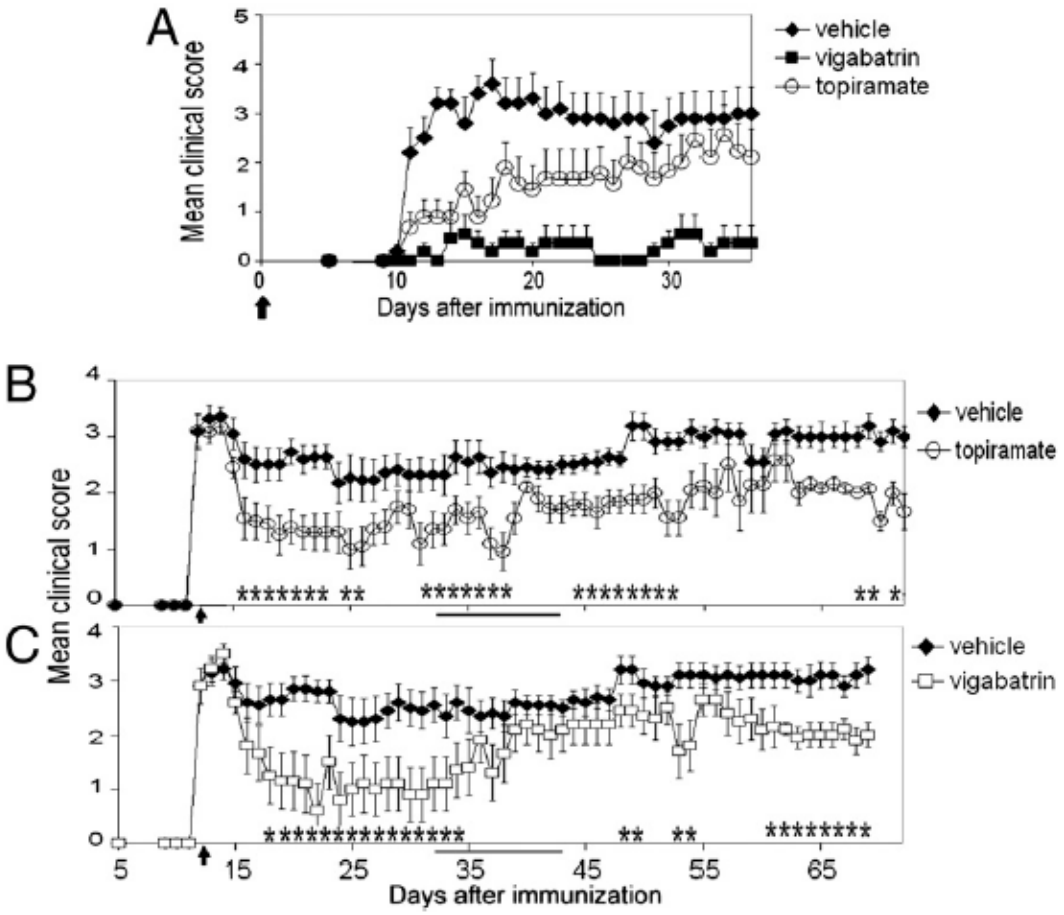




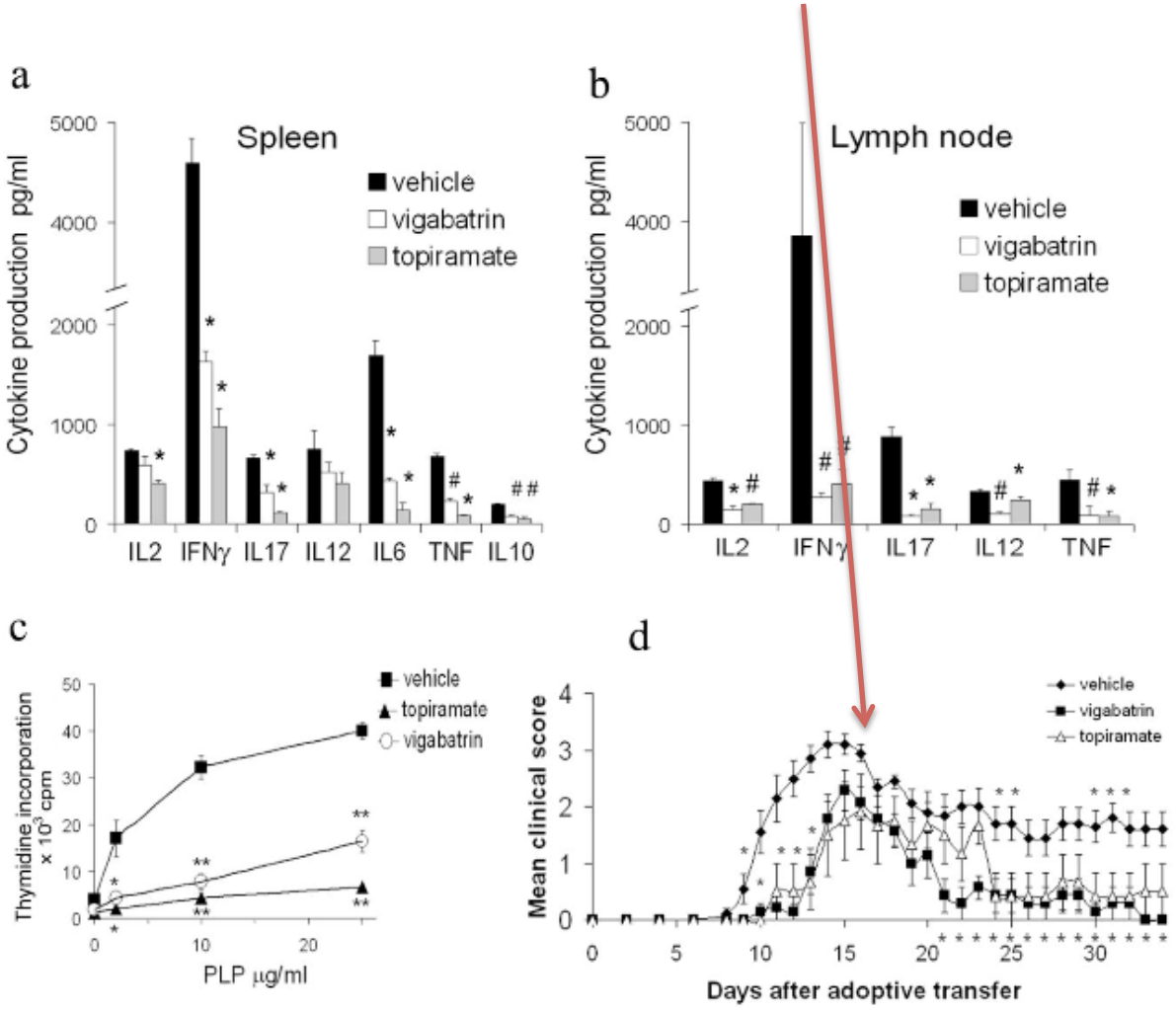
Splenocytes, T cells and Macrophages, activated in vitro with various concentrations of Topamax



Prevention and Treatment of EAE via Modulation of GABA



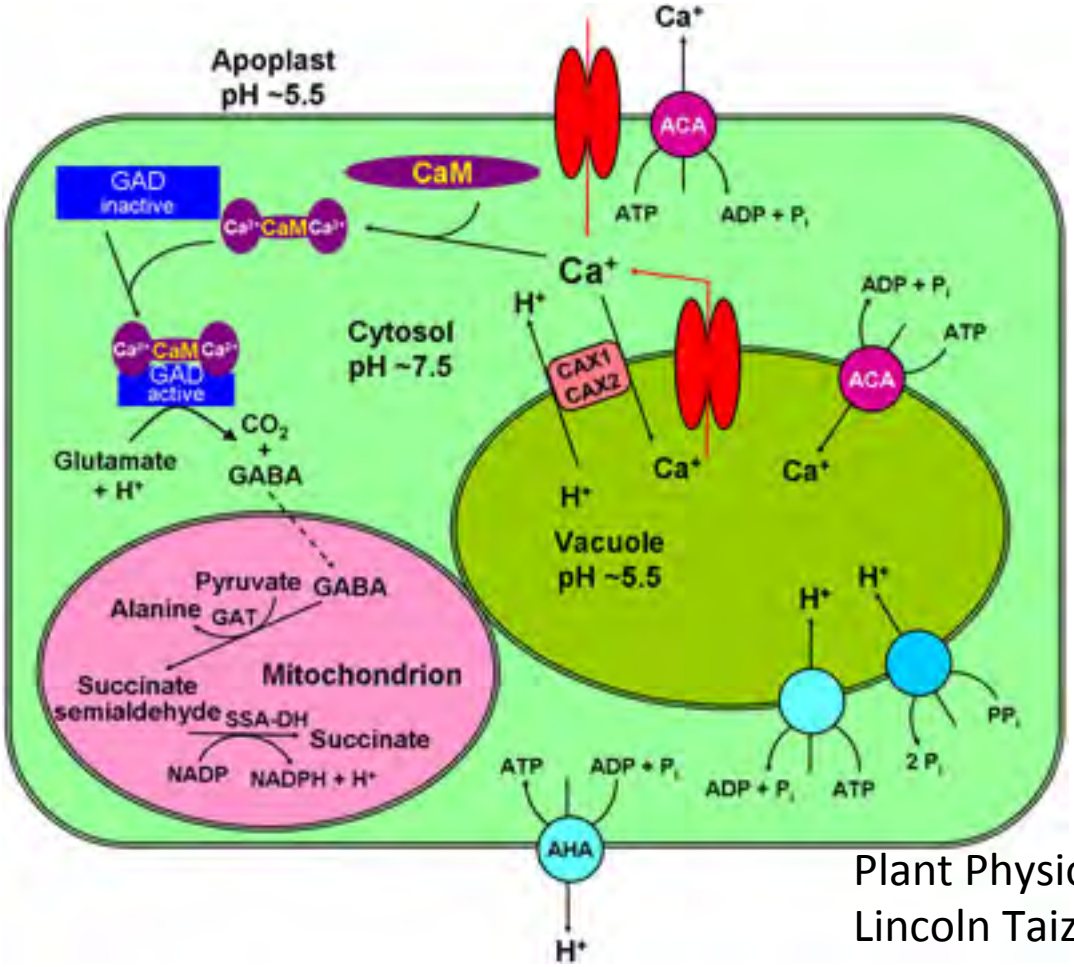
Isolation of Effect to Immune System

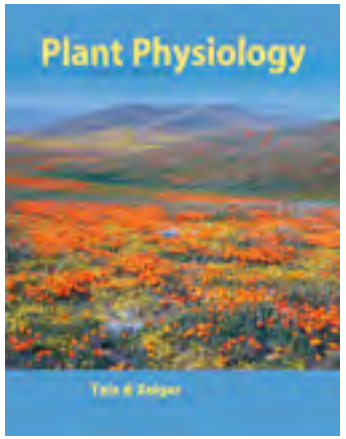


Ancient Role for GABA

One of the metabolic adaptations that plants make to heat stress leads to the accumulation GABA.

During heat stress, GABA accumulates to levels six- to tenfold higher than in unstressed plants.





Plant Physiology, Fourth Edition
Lincoln Taiz and Eduardo Zeiger

Butte & Colleagues
Science Transl. Med, Aug. 2011

RESEARCH ARTICLE

DRUG DISCOVERY

Computational Repositioning of the Anticonvulsant Topiramate for Inflammatory Bowel Disease

Joel T. Dudley,[1,2,3]* Marina Sirota,[1,2,3]* Mohan Shenoy,[4] Reetesh K. Pai,[5] Silke Roedder,[1,3] Annie P. Chiang,[1,2,3] Alex A. Morgan,[1,2,3] Minnie M. Sarwal,[1,3] Pankaj Jay Pasricha,[4] Atul J. Butte[1,3]†

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract for which there are few safe and effective therapeutic options for long-term treatment and disease maintenance. Here, we applied a computational approach to discover new drug therapies for IBD in silico, using publicly available molecular data reporting gene expression in IBD samples and 164 small-molecule drug compounds. Among the top compounds predicted to be therapeutic for IBD by our approach were prednisolone, a corticosteroid used to treat IBD, and topiramate, an anticonvulsant drug not previously described to have efficacy for IBD or any related disorders of inflammation or the gastrointestinal tract. Using a trinitrobenzenesulfonic acid (TNBS)–induced rodent model of IBD, we experimentally validated our topiramate prediction in vivo. Oral administration of topiramate significantly reduced gross pathological signs and microscopic damage in primary affected colon tissue in the TNBS-induced rodent model of IBD. These findings suggest that topiramate might serve as a therapeutic option for IBD in humans and support the use of public molecular data and computational approaches to discover new therapeutic options for disease.

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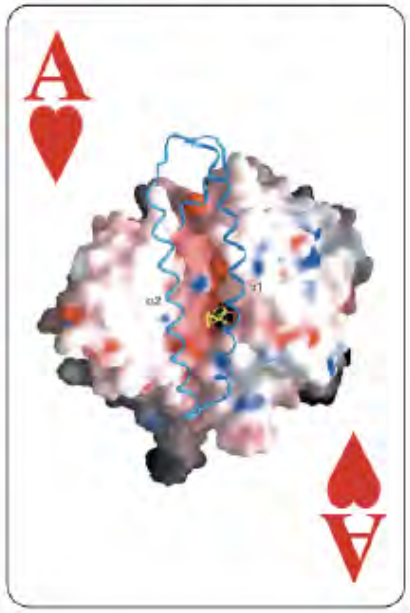
1. The inhibitory neurotransmitter GABA is immune suppressive
2. **Angiotensin Receptors are in MS Lesions**
   ACE inhibition is beneficial in animal models
3. **Immune suppressive lipids in the myelin sheath**

Blocking angiotensin-converting enzyme induces potent regulatory T cells and modulates TH1- and TH17-mediated autoimmunity

Michael Platten[a,b,1,2], Sawsan Youssef[a,1], Eun Mi Hur[a], Peggy P. Ho[a], May H. Han[a], Tobias V. Lanz[a], Lori K. Phillips[a], Matthew J. Goldstein[a], Roopa Bhat[a], Cedric S. Raine[c], Raymond A. Sobel[d], and Lawrence Steinman[a,2]

- Re-purposing Drugs: Topiramate, ACE Inhibitors, Statins

A hole in the Ace



Why not set expectations high after BG-12/Tecfidera?
Add-on Combo Might Be INEXPENSIVE!

PNAS

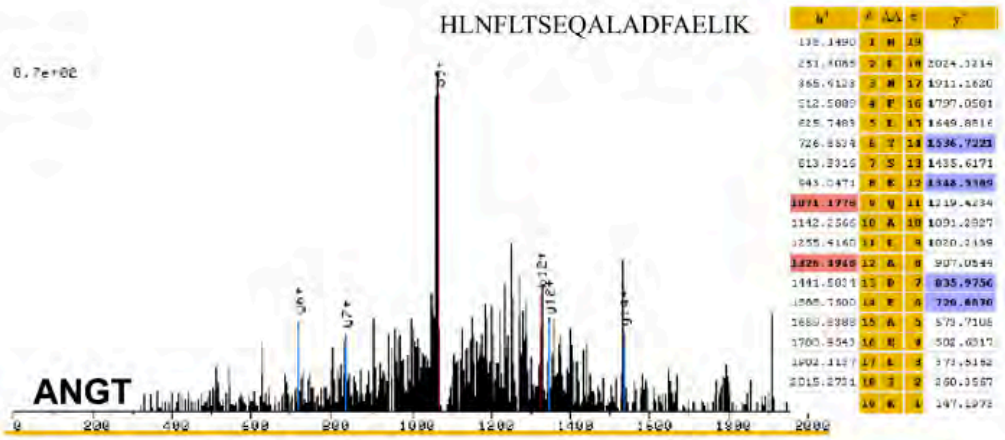
Role of the renin-angiotensin system in autoimmune inflammation of the central nervous system

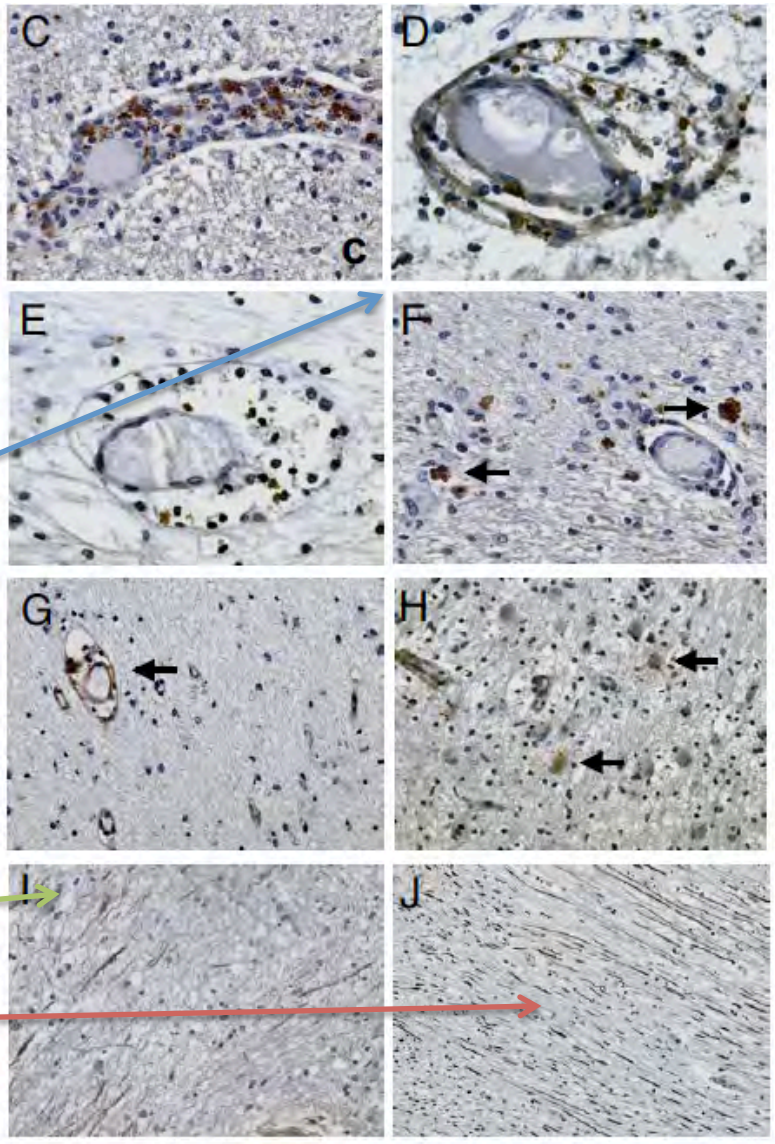
Johannes Stegbauer[a,1], De-Hyung Lee[b,1], Silvia Seubert[b], Gisa Ellrichmann[b], Arndt Manzel[b], Heda Kvakan[c], Dominik N. Muller[c], Stefanie Gaupp[b], Lars Christian Rump[a], Ralf Gold[b], and Ralf A. Linker[b,2]

[a]Department of Nephrology, Heinrich-Heine-University Düsseldorf, 40225 Düsseldorf, Germany; [b]Department of Neurology, St. Josef-Hospital, Ruhr-University Bochum, D-44791 Bochum, Germany; and [c]Max-Delbrück-Center for Molecular Medicine and Experimental and Clinical Research Center, Berlin 13125 Berlin, Germany

Edited by Jack L. Strominger, Harvard University, Cambridge, MA, and approved July 14, 2009 (received for review April 2, 2009)

2008 Proteomic Study Revealed Angiotensin in Acute Lesions





cord (*A*) or white matter from a patient with Alzheimer disease (*B*). Strong AT1R expression is detected in perivascular cuffs of a chronic active MS plaque (*C–F*), particularly in foamy macrophages (*F*, arrows). CD3 staining (*E*) of a neighbouring section of *D* shows presence of T cells. AT1R is also detectable in endothelial cells (*G*, arrow), astrocytes (*H*, arrows), and axons (*I*) within a chronic inactive MS plaque. AT1R is also strongly expressed in axons during viral encephalitis (*J*), suggesting that inflammation itself may drive neuronal AT1R expression. Magnifications: 20× (*J*), 40× (*A* and *I*), 60× (C, *F–H*), and 90× (*D* and *E*).

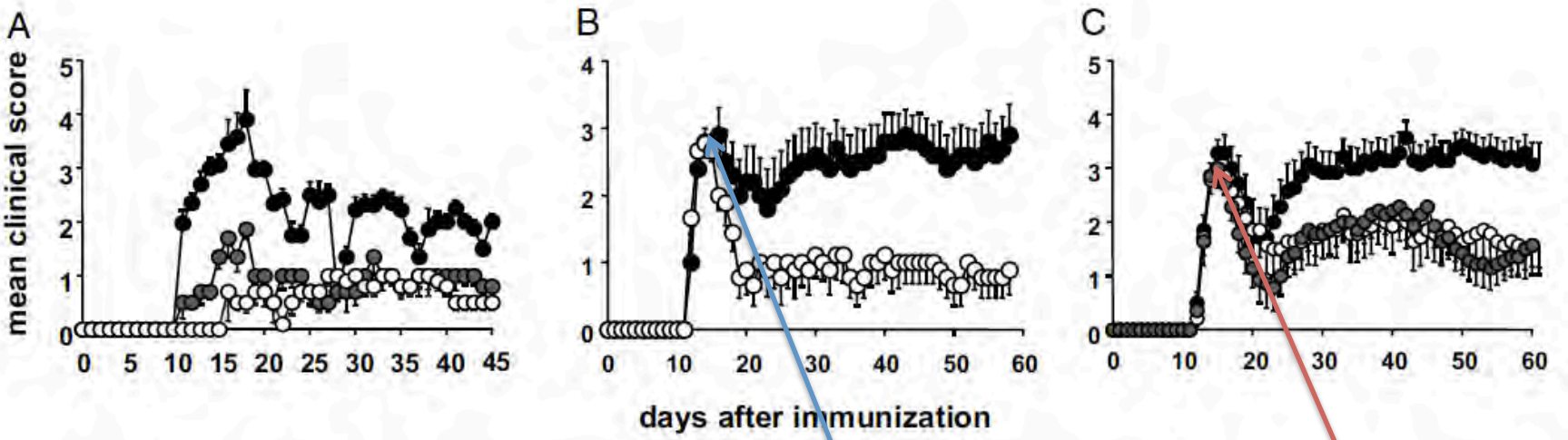


Fig. 6. Modulation of EAE by suppressing AII production or blocking AT1R. (*A*) Prevention of EAE after PLP immunization by lisinopril at 1 mg/kg/day (gray circles) or 10 mg/kg/day (open circles) compared with vehicle-treated controls (black circles), $n = 12$ per group. Treatment was initiated 2 days before immunization. Values are displayed as mean clinical scores as in Fig. 3. (*B*) Treatment of EAE after PLP immunization with lisinopril at 10 mg/kg/day (open circles) with vehicle controls (black circles), $n = 15$ per group. Treatment was initiated at the peak of first clinical disease activity (day 15 after immunization). Values are displayed as mean clinical scores. (*C*) Treatment of EAE after PLP immunization with lisinopril at 10 mg/kg/day (open circles) or candesartan at 1 mg/kg/day (gray circles) compared with vehicle controls (black circles), $n = 15$ per group. Treatment was initiated at the peak of first clinical disease activity (day 15 after immunization). Values are displayed as mean clinical scores. (*D*) H&E stained spinal cord sections of SJL/J mice with

transparency life sciences

Transparency Life Sciences Obtains Exclusive Option from Stanford University for use of Lisinopril in Multiple Sclerosis

Like Tweet Share

- SAB Chair Steinman Presents Preclinical Data on Potential of ACE Inhibitor Lisinopril to Treat MS at Gordon Conference -

- Lisinopril MS Protocol is First to Use Crowdsourced Web Platform Allowing Patients, Physicians, Researchers and Others to Participate in Clinical Trial Design -

NEW YORK, March 5, 2012 /PRNewswire/ -- Transparency Life Sciences, LLC (TLS) the world's first drug development company based on open innovation and crowdsourcing, today announced that it has concluded an agreement with Stanford University giving the company an exclusive option to license intellectual property covering the use of lisinopril as a treatment for multiple sclerosis (MS). Separately, TLS announced that MS expert Dr. Lawrence Steinman, the George A. Zimmermann Professor of Neurology and Neurological Sciences & Pediatrics at the Stanford School of Medicine and Chair of the TLS Scientific Advisory Board, presented preclinical data on the potential of lisinopril in MS at a recent Gordon Conference.

Statins

The HMG-CoA reductase inhibitor, atorvastatin, promotes a Th2 bias and reverses paralysis in central nervous system autoimmune disease

Sawsan Youssef*, Olaf Stüve†, Juan C. Patarroyo†, Pedro J. Ruiz*‡, Jennifer L. Radosevich*, Eun Mi Hur*, Manuel Bravo†, Dennis J. Mitchell*, Raymond A. Sobel§, Lawrence Steinman* & Scott S. Zamvil†

Effect of high-dose simvastatin on brain atrophy and disability in secondary progressive multiple sclerosis (MS-STAT): a randomised, placebo-controlled, phase 2 trial

Jeremy Chataway, Nadine Schuerer, Ali Alsanousi, Dennis Chan, David MacManus, Kelvin Hunter, Val Anderson, Charles R M Bangham, Shona Clegg, Casper Nielsen, Nick C Fox, David Wilkie, Jennifer M Nicholas, Virginia L Calder, John Greenwood, Chris Frost, Richard Nicholas

Summary

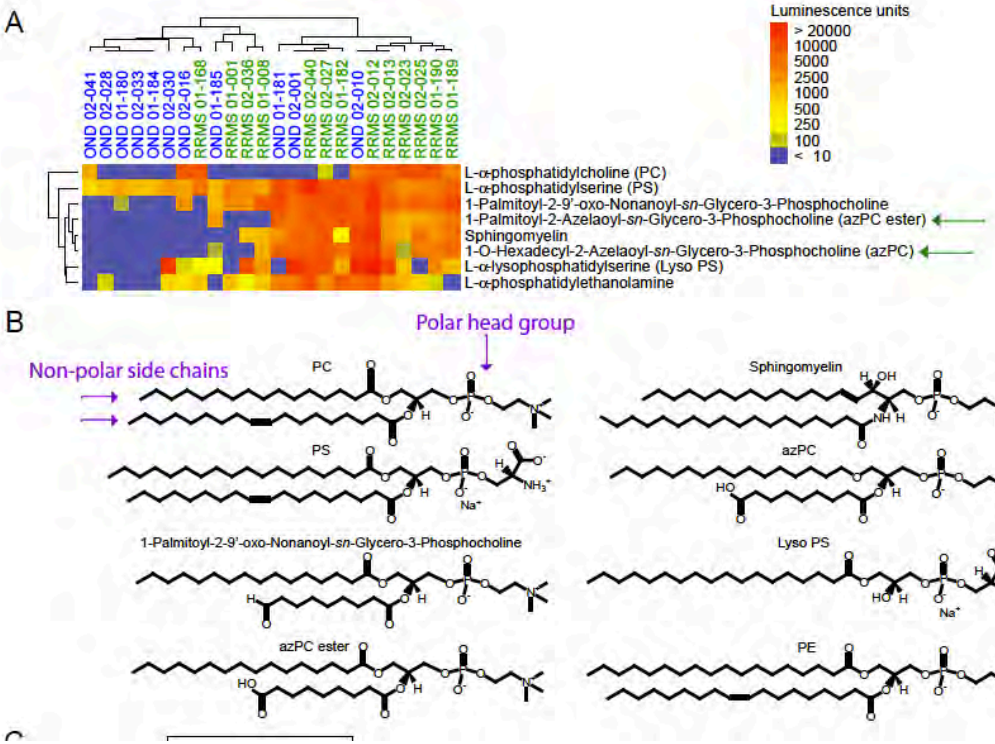
Background Secondary progressive multiple sclerosis, for which no satisfactory treatment presently exists, accounts for most of the disability in patients with multiple sclerosis. Simvastatin, which is widely used for treatment of vascular disease, with its excellent safety profile, has immunomodulatory and neuroprotective properties that could make it an appealing candidate drug for patients with secondary progressive multiple sclerosis.

Methods We undertook a double-blind, controlled trial between Jan 28, 2008, and Nov 4, 2011, at three neuroscience centres in the UK. Patients aged 18–65 years with secondary progressive multiple sclerosis were randomly assigned (1:1), by a centralised web-based service with a block size of eight, to receive either 80 mg of simvastatin or placebo. Patients, treating physicians, and outcome assessors were masked to treatment allocation. The primary outcome was the annualised rate of whole-brain atrophy measured from serial volumetric MRI. Analyses were by intention to treat and per protocol. This trial is registered with ClinicalTrials.gov, number NCT00647348.

Findings 140 participants were randomly assigned to receive either simvastatin (n=70) or placebo (n=70). The mean annualised atrophy rate was significantly lower in patients in the simvastatin group (0·288% per year [SD 0·521]) than in those in the placebo group (0·584% per year [0·498]). The adjusted difference in atrophy rate between groups was –0·254% per year (95% CI –0·422 to –0·087; p=0·003); a 43% reduction in annualised rate. Simvastatin was well tolerated, with no differences between the placebo and simvastatin groups in proportions of participants who had serious adverse events (14 [20%] *vs* nine [13%]).

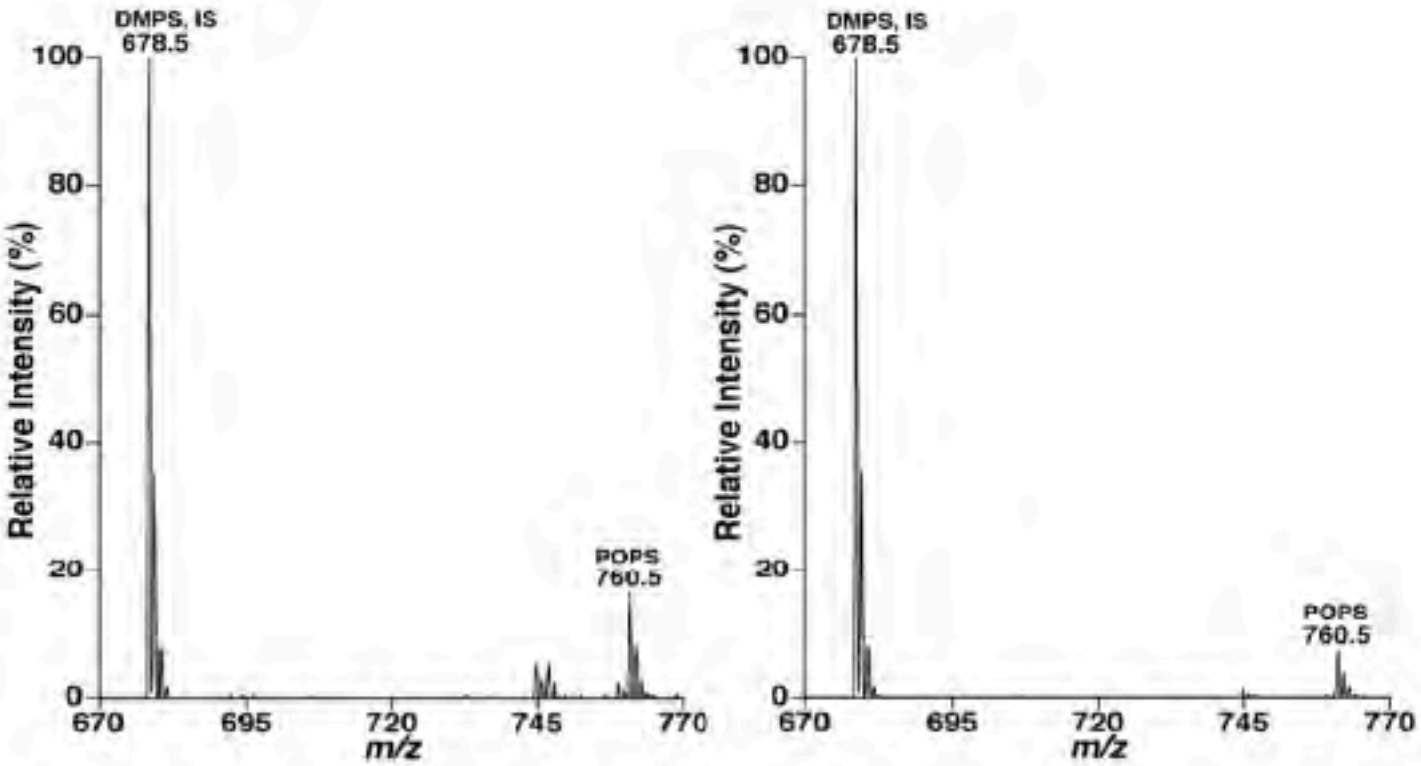
Interpretation High-dose simvastatin reduced the annualised rate of whole-brain atrophy compared with placebo, and was well tolerated and safe. These results support the advancement of this treatment to phase 3 testing.

Identification of Naturally Occurring Fatty Acids of the Myelin Sheath That Resolve Neuroinflammation



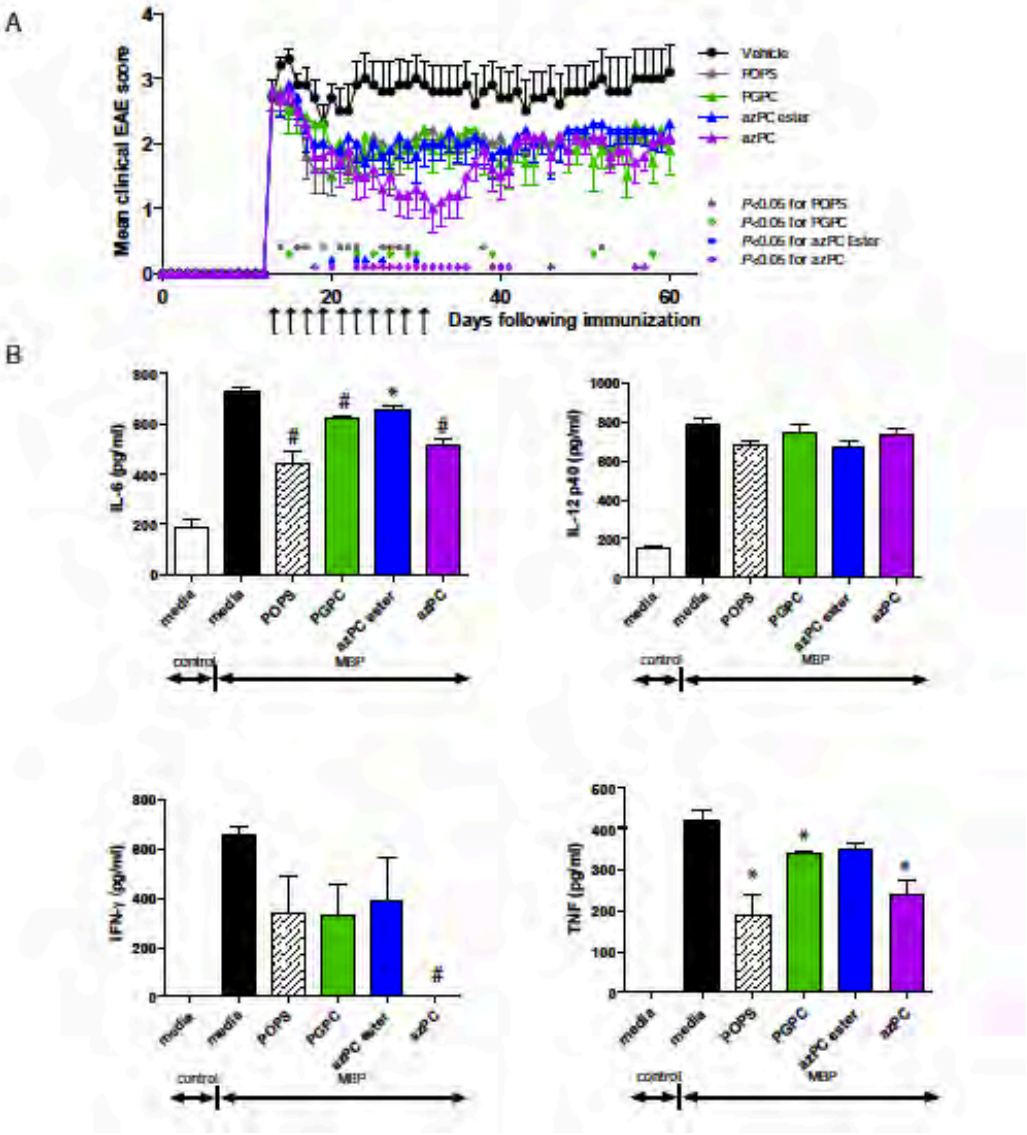
Science Translational Medicine, 2012

A



B

Species	Monoisotopic mass	Control 1	Control 2	MS 1	MS 2	Per mg protein
azPC	672.44	13.30	19.96	7.62	12.45	pmol
azPC ester	658.46	8.98	9.58	5.48	5.50	pmol
PGPC	616.38	19.98	16.03	5.23	14.06	pmol
POPS	760.51	0.67	1.89	0.61	0.51	nmol


A
Mean clinical EAE score
Days following immunization
Vehicle
POPS
PGPC
azPC ester
azPC
P<0.05 for POPS
P<0.05 for PGPC
P<0.05 for azPC Ester
P<0.05 for azPC
B
IL-6 (pg/ml)
IL-12 p40 (pg/ml)
IFN-γ (pg/ml)
TNF (pg/ml)
media
POPS
PGPC
azPC ester
azPC
control
MBP

In the next 50 minutes I shall share with you some "tractable" targets derived from various "omics", without the aid of "genomics". I shall describe the following:

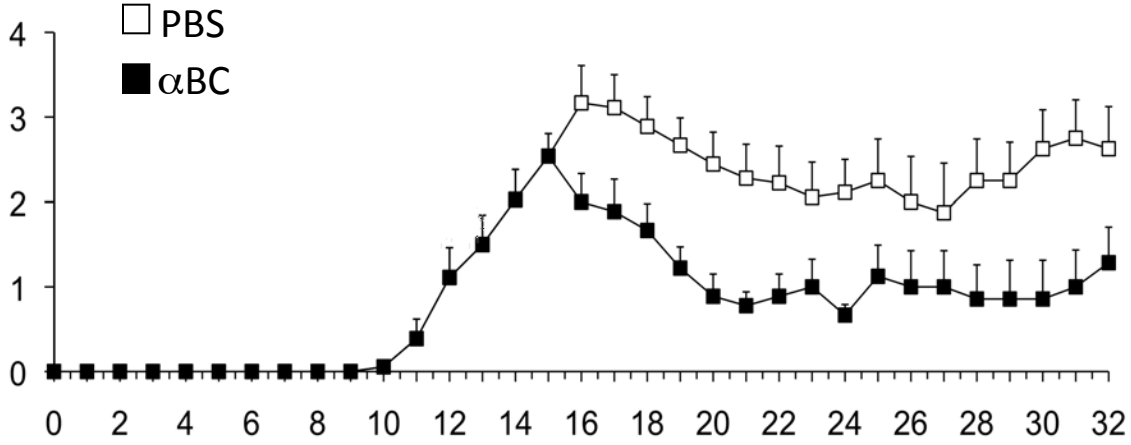
1. The inhibitory neurotransmitter GABA is immune suppressive
2. Angiotensin Receptors are in MS Lesions
   ACE inhibition is beneficial in animal models
3. Immune suppressive lipids in the myelin sheath
4. **Infamous amyloid proteins provide protection, not harm in neuroinflammatory conditions**

nature

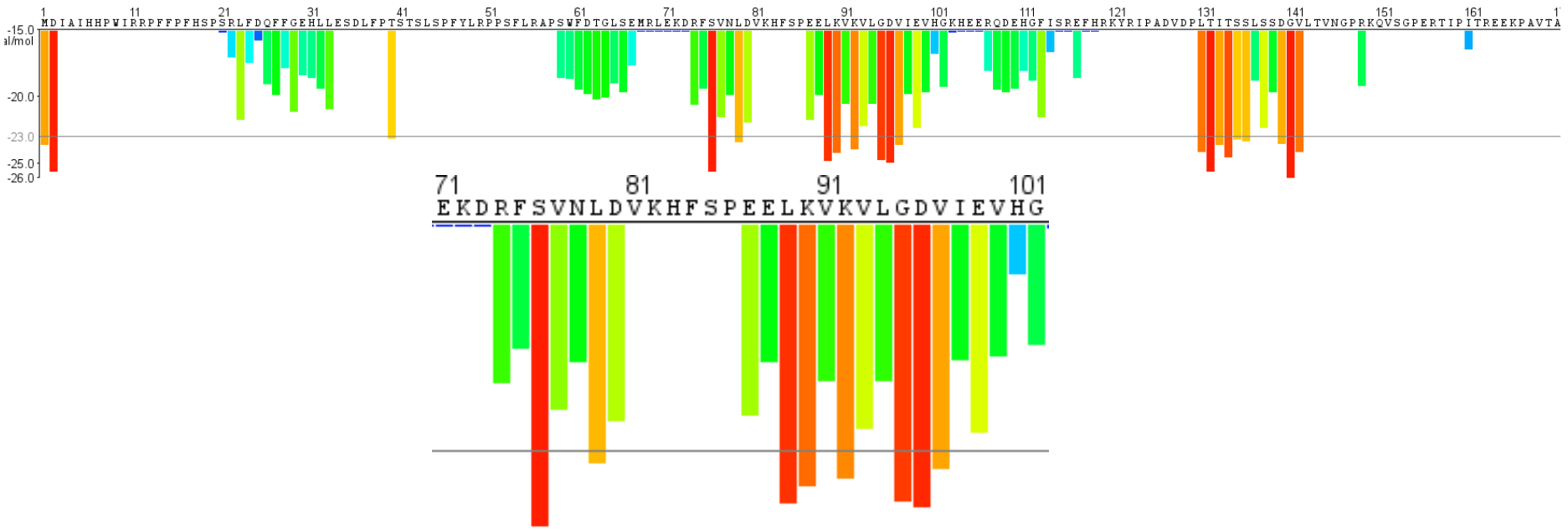
LETTERS

Protective and therapeutic role for αB-crystallin in autoimmune demyelination

Shalina S. Ousman[1], Beren H. Tomooka[2,3], Johannes M. van Noort[4], Eric F. Wawrousek[5], Kevin O'Conner[6], David A. Hafler[6], Raymond A. Sobel', William H. Robinson[2,3] & Lawrence Steinman[1]



Regions from alpha B crystallin predicted to form fibrils



- Six amino acids is minimum needed to form a beta sheet
- Zipperdb is a website by Eisenberg's group at UCLA
- Algorithm to predict segments with high fibrillation propensity that could form a "steric zipper" → two self-complementary beta sheets resulting in the spine of an amyloid fibril (Thompson et al., PNAS, 2006).
- Its Rosetta energy is utilized to determine propensity to form amyloid fibrils; -23 kcal/mol was chosen as the threshold.

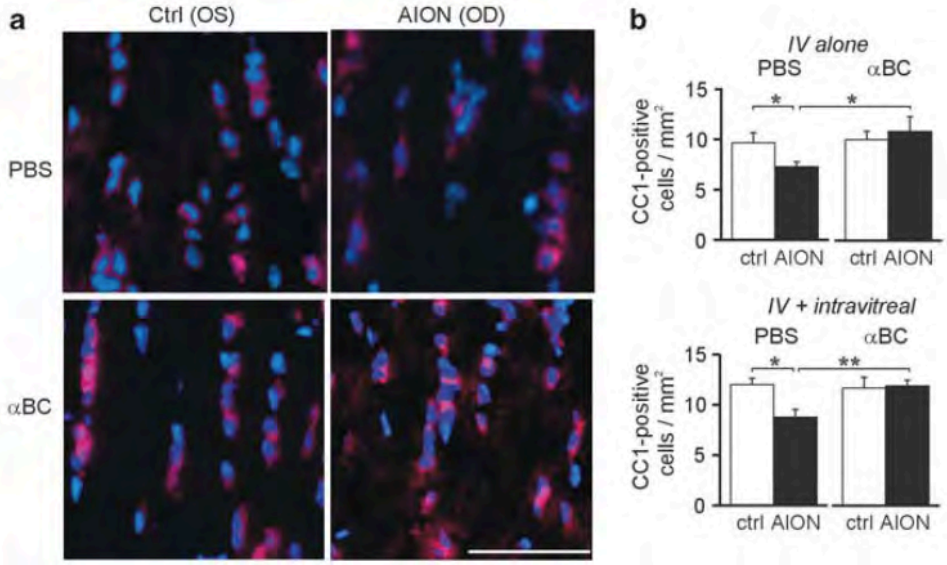
Summary

What was known before

- The αB-crystallin is an important molecule in inflammatic and ischemia and is upregulated following different brai and eye injury models.
- Intravitreal treatment with αB-crystallin improved optic nerve crush model.
- Treatment with αB-crystallin improved animal model of multiple sclerosis.

What this study adds

- The αB-crystallin is upregulated rapidly following experimental anterior ischemic optic neuropathy.
- Treatment with αB-crystllin enhanced optic nerve functio as shown by improvement in the latency of visually evok responses. This was done with serial intracranial visual evoked potentials.
- The αB-crystallin improved optic nerve function by complete rescue of optic nerve oligodendrocytes followin experimental anterior ischemic optic neuropathy.



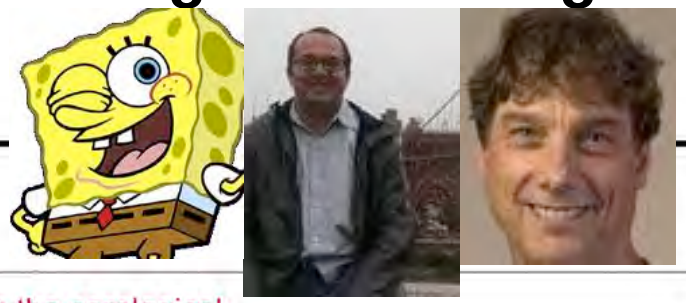
Eye (2011), 1–9

www.nature.com/eye

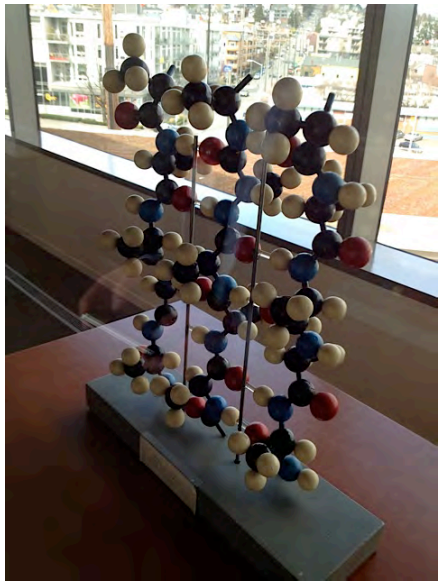
Functional rescue of experimental ischemic optic neuropathy with αB-crystallin

S Pangratz-Fuehrer[1], K Kaur[1], SS Ousman[2], L Steinman[2] and YJ Liao[1]

Plasma Protein Concentrations Range over 12 logs



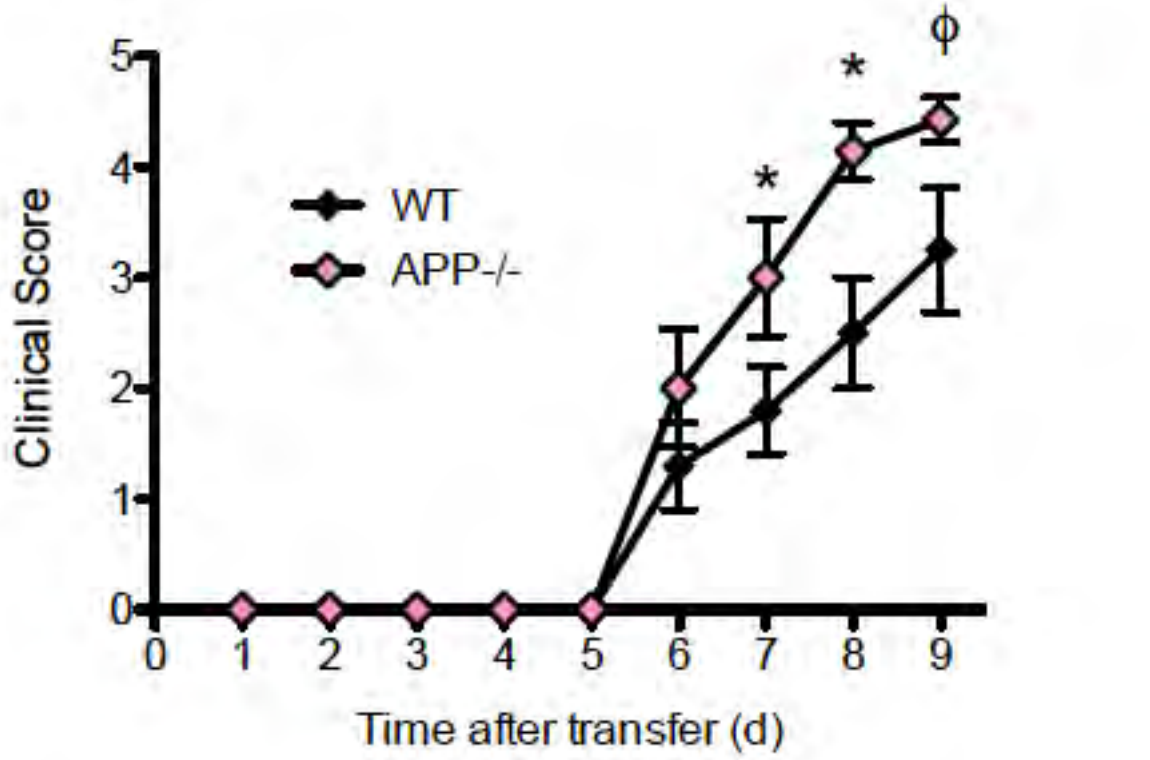
Novel Guardian Amyloid Proteins in MS: Cryab, APP, Tau, Prp



Loss of Function Experiments with Amyloid Proteins

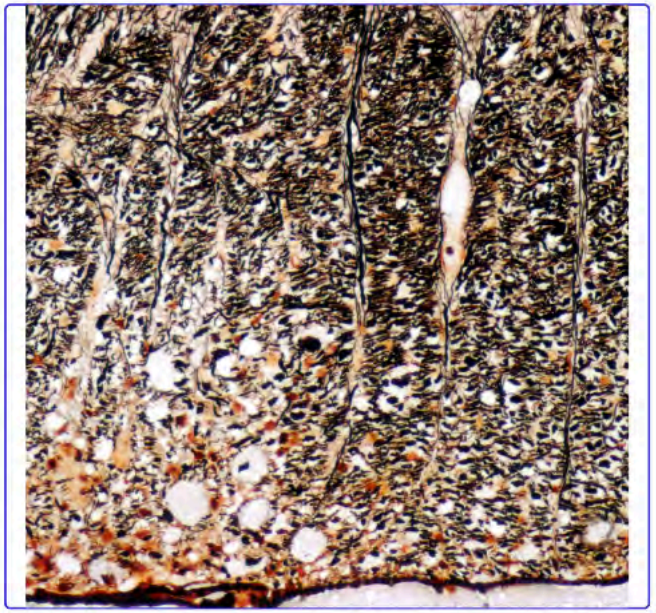
Disease Exacerbation in APP-/-

Concordant Loss of Function Expts



EAE Worse in PrPc-/-

JNI JOURNAL OF NEUROINFLAMMATION



Exacerbation of experimental autoimmune encephalomyelitis in prion protein (PrPc)-null mice: evidence for a critical role of the central nervous system

Gourdain *et al.*

BioMed Central

Gourdain *et al. Journal of Neuroinflammation* 2012, **9**:25
http://www.jneuroinflammation.com/content/9/1/25 (26 January 2012)

EAE Worse in Tau-/-

J Neuropathol Exp Neurol

Vol. 71, No. 5
May 2012
pp. 422–433

ORIGINAL ARTICLE

Mice Devoid of Tau Have Increased Susceptibility to Neuronal Damage in Myelin Oligodendrocyte Glycoprotein-Induced Experimental Autoimmune Encephalomyelitis

Jason G. Weinger, PhD, Peter Davies, PhD, Christopher M. Acker, BS, Celia F. Brosnan, PhD, Vladislav Tsiperson, PhD, Ashrei Bayewitz, BS, and Bridget Shafit-Zagardo, PhD

EAE Worse in SAP-/-

Immunology and Cell Biology (2012) 90, 388–395

www.nature.com/icb

ORIGINAL ARTICLE

SAP suppresses the development of experimental autoimmune encephalomyelitis in C57BL/6 mice

Zhe Ji[1,2], Zun-Ji Ke[2] and Jian-Guo Geng[1]

Experimental autoimmune encephalomyelitis (EAE) is a CD4+ T cell-mediated disease of the central nervous system. Serum amyloid P component (SAP) is a highly conserved plasma protein named for its universal presence in amyloid deposits. Here we report that SAP-transgenic mice had unexpectedly attenuated EAE due to impaired encephalitogenic responses. Following induction with myelin oligodendroglial glycoprotein (MOG) peptide 35–55 in complete Freund's adjuvant, SAP-transgenic mice showed reduced spinal cord inflammation with lower severity of EAE attacks as compared with control C57BL/6 mice. However, in SAP-Knockout mice, the severity of EAE is enhanced. Adoptive transfer of Ag-restimulated T cells from wild type to SAP-transgenic mice, or transfer of SAP-transgenic Ag-restimulated T cells to control mice, induced milder EAE. T cells from MOG-primed SAP-transgenic mice showed weak proliferative responses. Furthermore, in SAP-transgenic mice, there is little infiltration of CD45-positive cells in the spinal cord. *In vitro*, SAP suppressed the secretion of interleukin-2 stimulated by P-selectin and blocked P-selectin binding to T cells. Moreover, SAP could change the affinity between α4-integrin and T cells. These data suggested that SAP could antagonize the development of the acute phase of inflammation accompanying EAE by modulating the function of P-selectin.
Immunology and Cell Biology (2012) **90**, 388–395; doi:10.1038/icb.2011.51; published online 7 June 2011

Worsened Brain Trauma in APP-/-

Journal of Neurochemistry JNC

JOURNAL OF NEUROCHEMISTRY | 2012 | 122 | 208–220 doi: 10.1111/j.1471-4159.2012.07761.x

ORIGINAL ARTICLE

sAPPα rescues deficits in amyloid precursor protein knockout mice following focal traumatic brain injury

Frances Corrigan,*,† Robert Vink,*,† Peter C. Blumbergs,*,† Colin L. Masters,‡ Roberto Cappai§,[1] and Corinna van den Heuvel*,†,[1]

*Discipline of Anatomy and Pathology, School of Medical Sciences, University of Adelaide, Adelaide South Australia, Australia

†Centre for Neurological Diseases, Hanson Institute, South Australia, Australia

‡Mental Health Research Institute, University of Melbourne, Victoria, Australia

§Department of Pathology and Bio21 Molecular Science and BioTechnology Institute, The University of Melbourne, Victoria, Australia

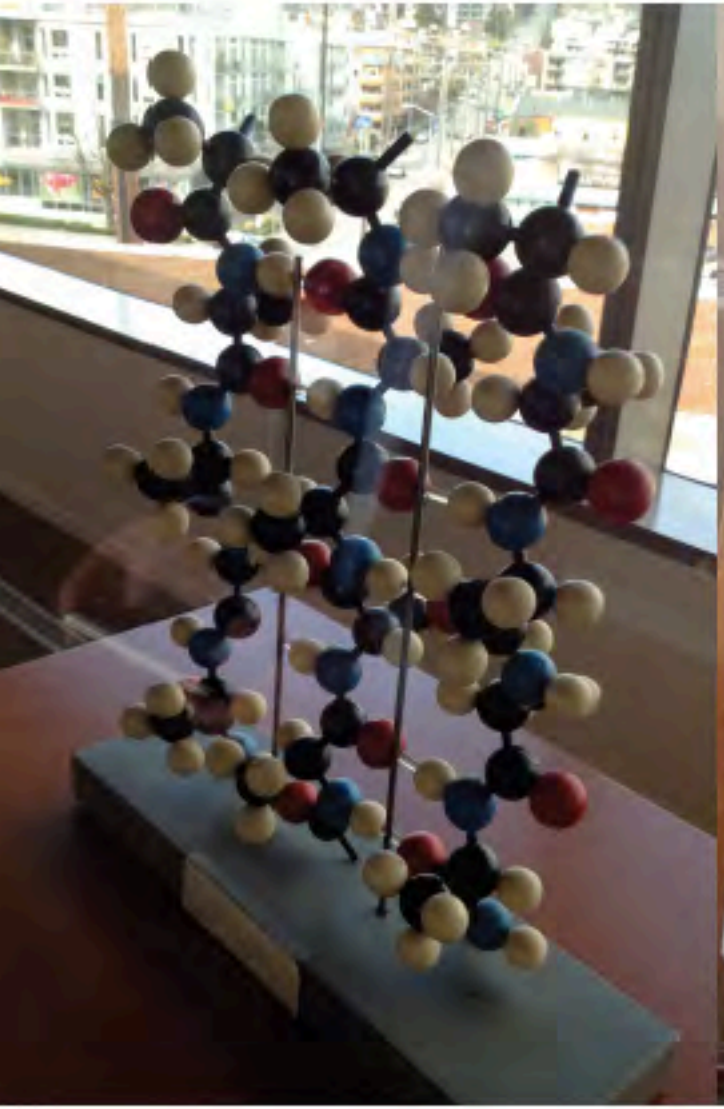
Abstract

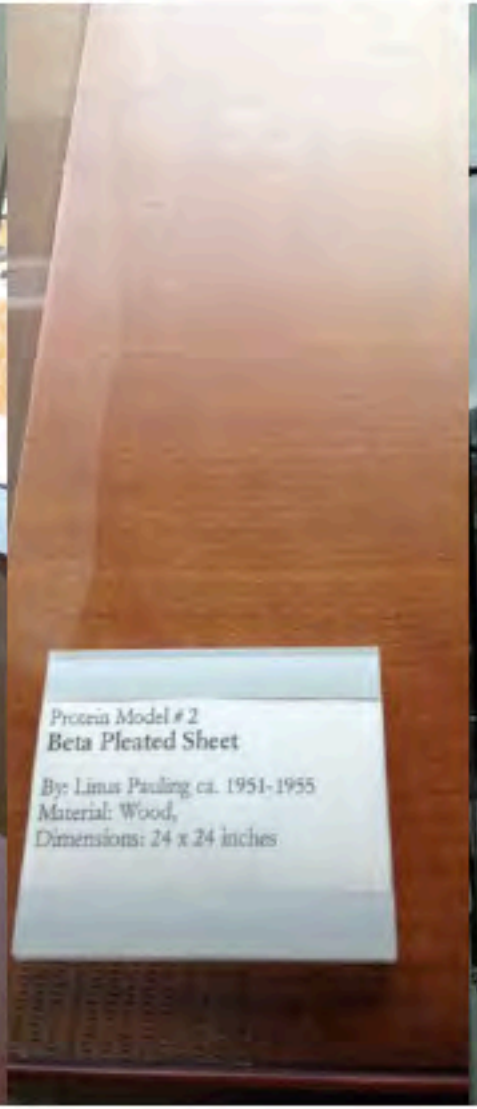
The amyloid precursor protein (APP) is thought to be neuroprotective following traumatic brain injury (TBI), although definitive evidence at moderate to severe levels of injury is lacking. In the current study, we investigated histological and functional outcomes in APP−/− mice compared with APP+/+ mice following a moderate focal injury, and whether administration of sAPPα restored the outcomes in knockout animals back to the wildtype state. Following moderate controlled cortical impact injury, APP−/− mice demonstrated greater impairment in motor and cognitive outcome as determined by the ledged beam and Barnes Maze tests respectively ($p < 0.05$). This corresponded with the degree of neuronal damage, with APP−/− mice having significantly greater lesion volume (25.0 ± 1.6 vs. 20.3 ± 1.6%, $p < 0.01$) and hippocampal damage, with less remaining CA neurons (839 ± 245 vs. 1353 ± 142 and 1401 ± 263). This was also associated with an impaired neuroreparative response, with decreased GAP-43 immunoreactivity within the cortex around the lesion edge compared with APP+/+ mice. The deficits observed in the APP−/− mice related to a lack of sAPPα, as treatment with exogenously added sAPPα post-injury improved APP−/− mice histological and functional outcome to the point that they were no longer significantly different to APP+/+ mice ($p < 0.05$). This study shows that endogenous APP is potentially protective at moderate levels of TBI, and that this neuroprotective activity is related to the presence of sAPPα. Importantly, it indicates that the mechanism of action of exogenously added sAPPα is independent of the presence of endogenous APP.

Keywords: amyloid precursor protein, sAPPα, traumatic brain injury.

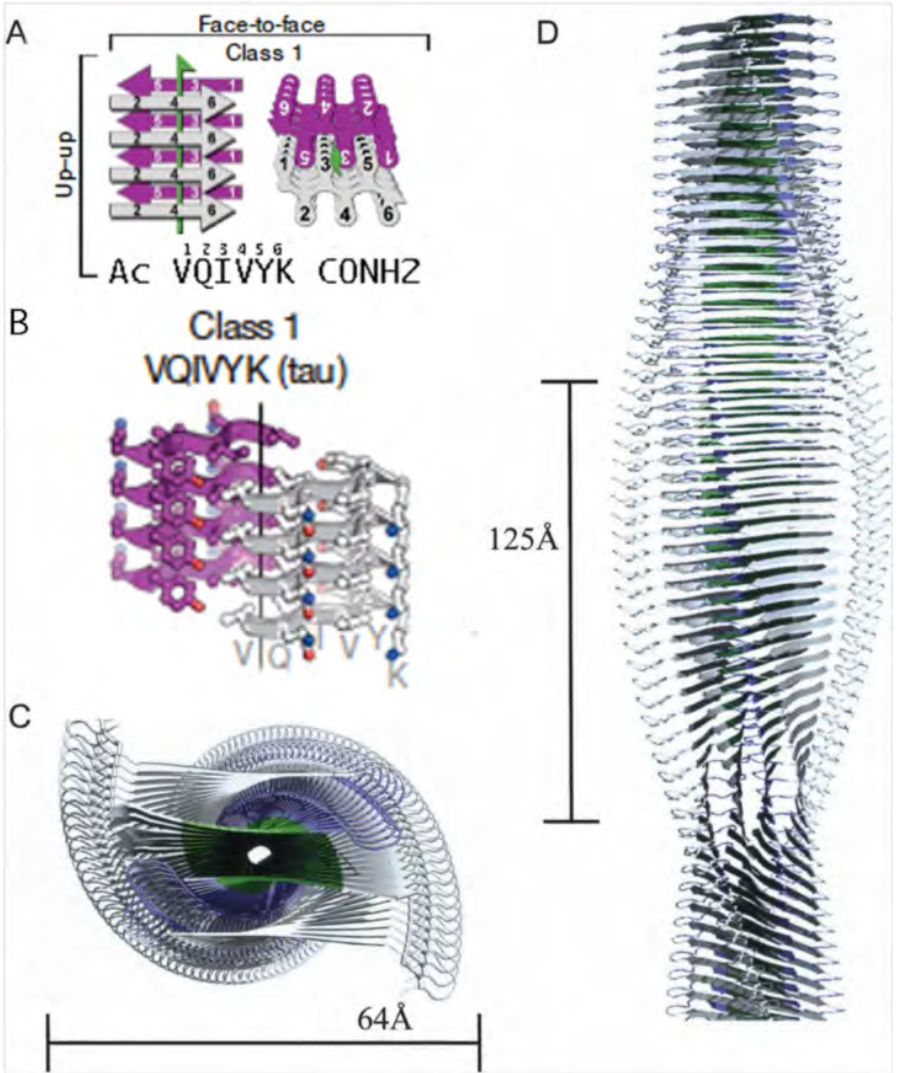
J. Neurochem. (2012) **122**, 208–220.

Linus Pauling Model β-Sheet Gates Headquarters, Seattle





Dominant feature of amyloid fibrils
cross beta spine
replicated by the zipper interface formed with peptides as short as 6 amino acids

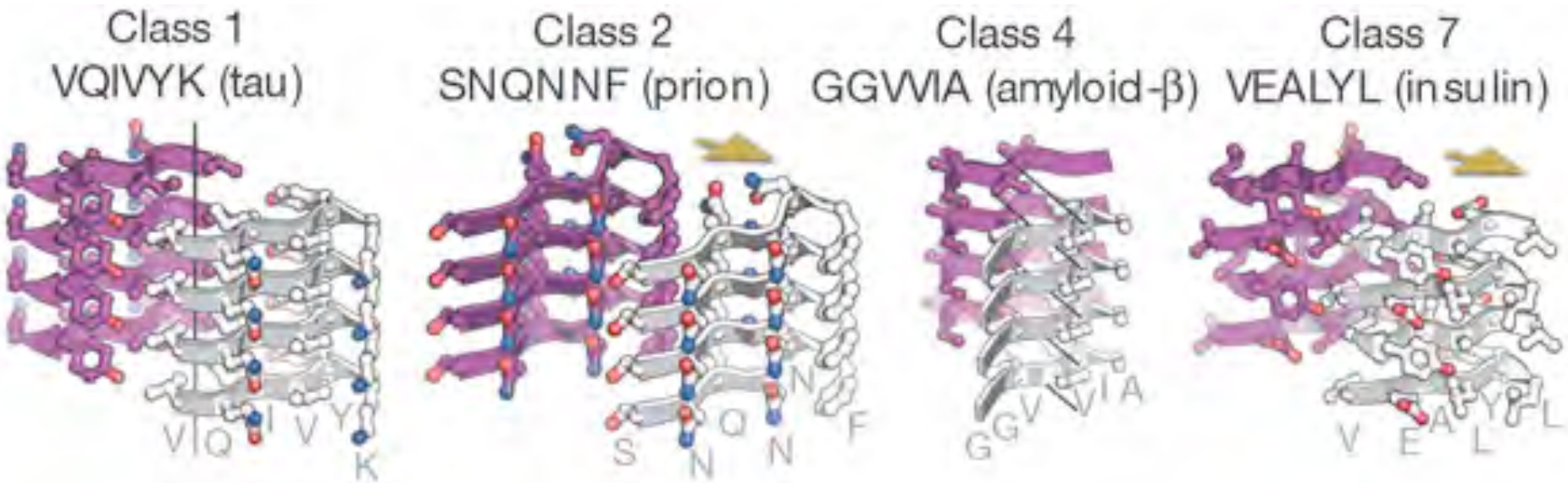


Atomic structures of amyloid cross-β spines reveal varied steric zippers

Michael R. Sawaya, Shilpa Sambashivan, Rebecca Nelson, Magdalena I. Ivanova, Stuart A. Sievers, Marcin I. Apostol, Michael J. Thompson, Melinda Balbirnie, Jed J. W. Wiltzius, Heather T. McFarlane, Anders Ø. Madsen, Christian Riekel & David Eisenberg

Nature 447, 453-457(24 May 2007)

3D views of representative steric zipper structures of classes 1, 2, 4 and 7, showing the front sheet in silver and the rear sheet in purple.



Geometry of Beta Zippers

									Mr	PI	water sol
HspB5 76-81 P02511		Ac	S	V	N	L	D	V	CONH2	688.35	3.80	X
Shuffle HspB5 76-81		Ac	V	D	N	L	V	S	CONH2	688.35	3.80	X
HspB5 89-94 P02511		Ac	L	K	V	K	V	L	CONH2	741.52	10.0	X
Tau 623 -628 P10636	face to face up-up anti	Ac	V	Q	I	V	Y	K	CONH2	791.47	8.6	X
Insulin beta 11-16 (36-41) P01308	face to back up-up anti	Ac	V	E	A	L	Y	L	CONH2	749.41	4.0	X
Insulin alpha 12-17(102-107) P01308	face to back up-up anti	Ac	L	Y	Q	L	E	N	CONH2	821.4	4.0	X
major prion protein148-153 (170-175) P04156	face to back up-up par	Ac	S	N	Q	N	N	F	CONH2	763	5.2	
amyloid beta A4 protein (706-711) (35-40) P05067	face to face up-down anti	Ac	M	V	G	G	V	V	CONH2	603.32	5.3	
amyloid beta A4 protein (708-713) (37-42) P05067	face to back up-down anti	Ac	G	G	V	V	I	A	CONH2	557.33	5.5	

Self-assembling hexapeptides form immunosuppressive amyloid fibrils effective in neuroinflammation

Take Home From This Section

- Amyloid fibrils composed of peptides as short as six amino acids are anti-inflammatory and therapeutic in experimental autoimmune encephalomyelitis (EAE)

- Amyloidogenic hexapeptides, oppositely from fibrils composed of larger peptides or proteins, do not form toxic structures

- The fibrils act as particles that associate with and activate B-1a cells and macrophages and induce the migration of these cells to lymph nodes, resulting in immune suppression

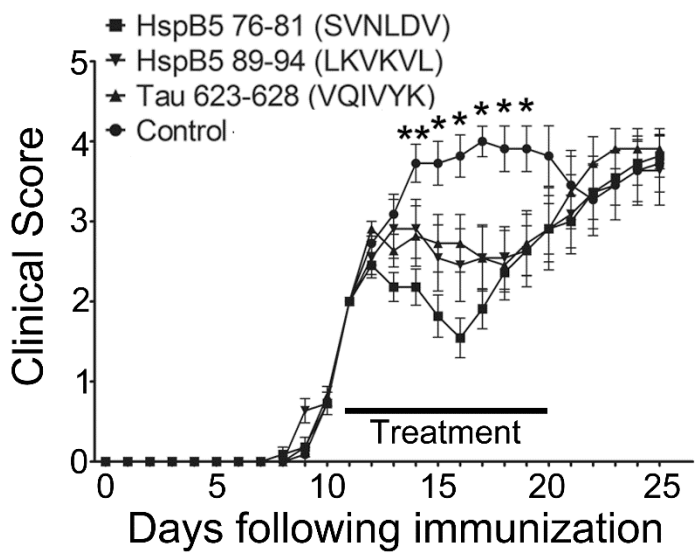
Hexapeptides tested in EAE

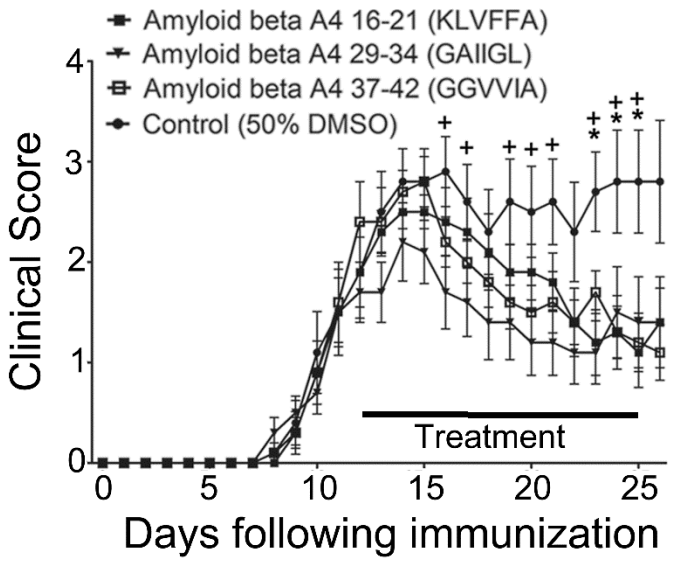
anionic, requires low pH									
HspB5 76-81	Ac	S	V	N	L	D	V	CONH2	
Insulin B chain 11-16	Ac	V	E	A	L	Y	L	CONH2	class 7
Insulin A chain 12-17	Ac	L	Y	Q	L	E	N	CONH2	class 7
cationic, requires high pH									
HspB5 89-94	Ac	L	K	V	K	V	L	CONH2	
Amyloid beta A4 protein 27-32	Ac	N	K	G	A	I	I	CONH2	class 1
cationic, readily form at all pH									
Tau 623-628	Ac	V	Q	I	V	Y	K	CONH2	class 1
Serum amyloid P 213-218	Ac	G	Y	V	I	I	K	CONH2	
Amyloid beta A4 protein 16-21	Ac	K	L	V	F	F	A	CONH2	class 7
Tau 623-628 D	Ac	v	q	i	v	y	k	CONH2	

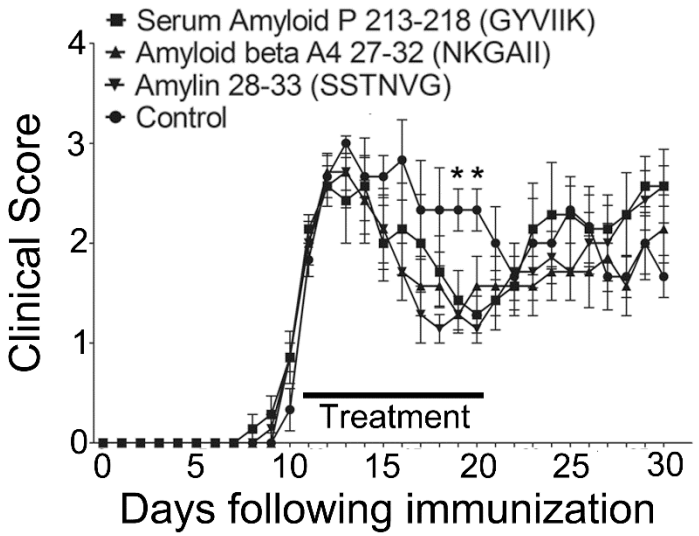
nonionizable polar									
Major prion protein 148-153	Ac	S	N	Q	N	N	F	CONH2	class
Apolipoprotein E 53-58	Ac	S	S	Q	V	T	Q	CONH2	
Amylin 28-33	Ac	S	S	T	N	V	G	CONH2	class
Ig Kappa chain 5-10	Ac	S	V	S	S	S	Y	CONH2	
nonionizable hydrophobic									
Amyloid beta A4 protein 29-34	Ac	G	A	I	I	G	L	CONH2	class
Amyloid beta A4 protein 35-40	Ac	M	V	G	G	V	V	CONH2	class
Amyloid beta A4 protein 37-42	Ac	G	G	V	V	I	A	CONH2	class
Amylin 24-29	Ac	G	A	I	L	S	S	CONH2	
Amyloid beta A4 protein 35-40 D	Ac	m	v	g	g	v	v	CONH2	

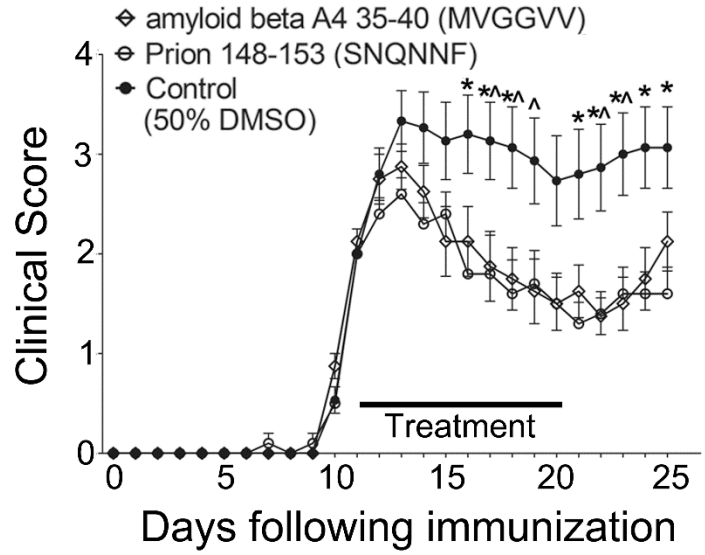
- Hexapeptides formed amyloid fibrils
  - As assessed by Thioflavin T staining
- Hexapeptides act as molecular chaperones
  - Determined by inhibition of denatured insulin aggregation

Hexapeptides are therapeutic in EAE







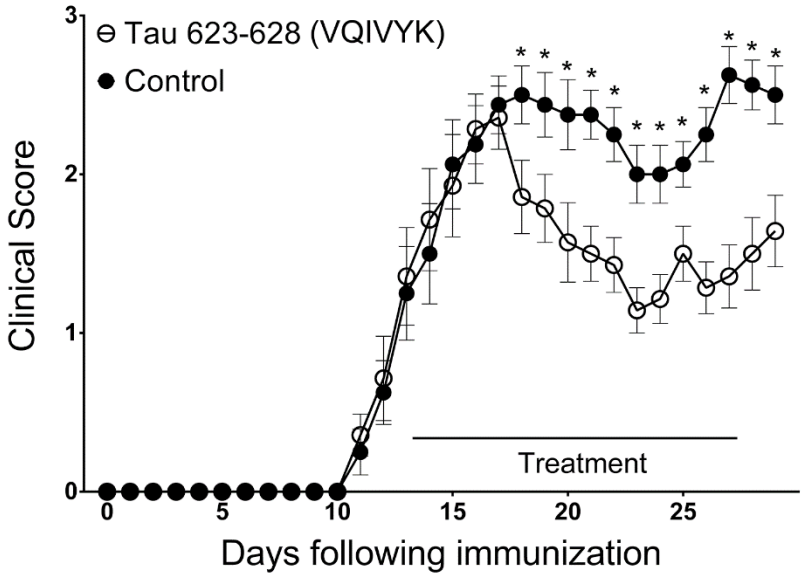


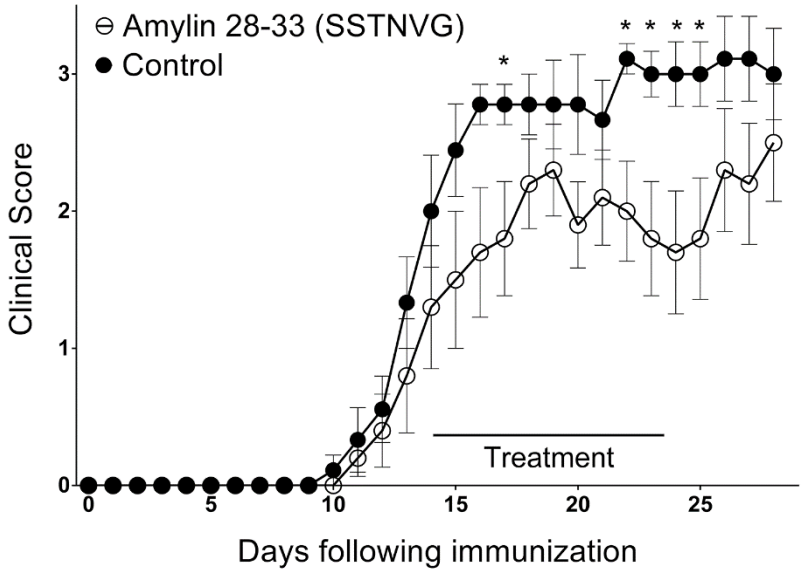
Science Translational Medicine AAAS

Amyloid Fibrils Composed of Hexameric Peptides Attenuate Neuroinflammation

Michael P. Kurnellas,[1] Chris M. Adams,[2] Raymond A. Sobel,[3] Lawrence Steinman,[1]* Jonathan B. Rothbard[1,4]

Sci Transl Med (2013); 5 (179): 179ra42.





- Act as molecular chaperones
- Bind pro-inflammatory mediators in the plasma
- Decrease pro-inflammatory cytokines in the plasma
- Reduce the number of inflammatory foci in the meninges and parenchyma of spinal cord and brain
- Not toxic to human monocytes

JEM

Mechanisms of action of therapeutic amyloidogenic hexapeptides in amelioration of inflammatory brain disease

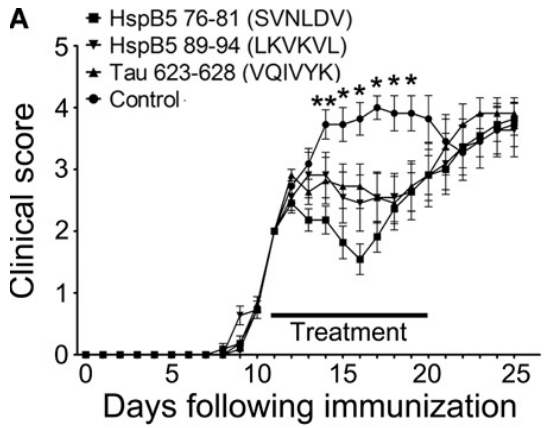
Michael P. Kurnellas, Jill M. Schartner, C. Garrison Fathman, Ann Jagger, Lawrence Steinman, Jonathan B. Rothbard

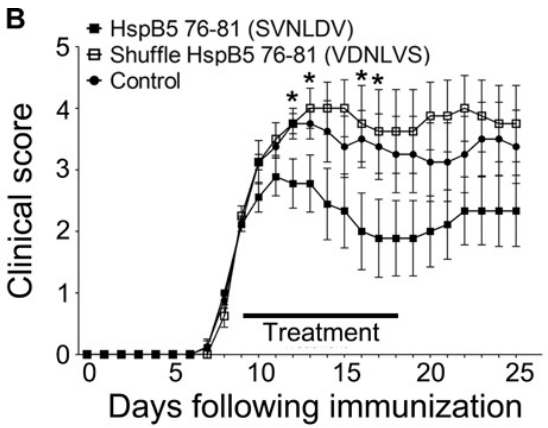
J Exp Med (2014); 211: 1847-56.

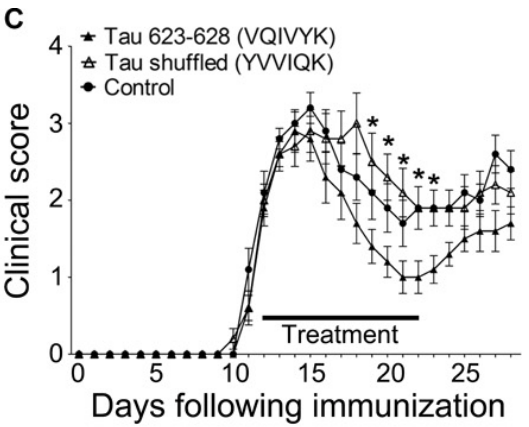
- Gene microarray analysis and qPCR confirmed a decrease in pro-inflammatory cytokines and revealed an induction of genes involved in the type 1 interferon pathway

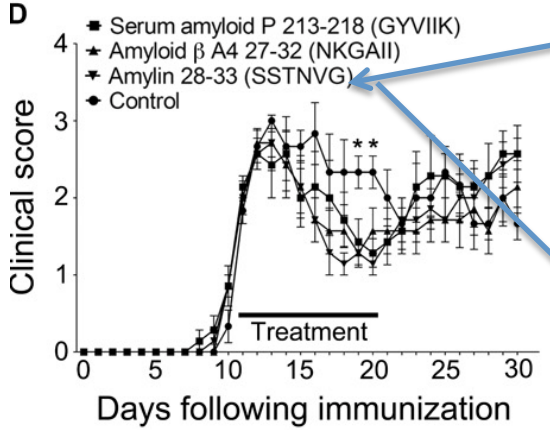
- Amyloidogenic peptides taken up by neutrophils resulted in NETosis, which activated pDCs leading to the induction of type I IFN

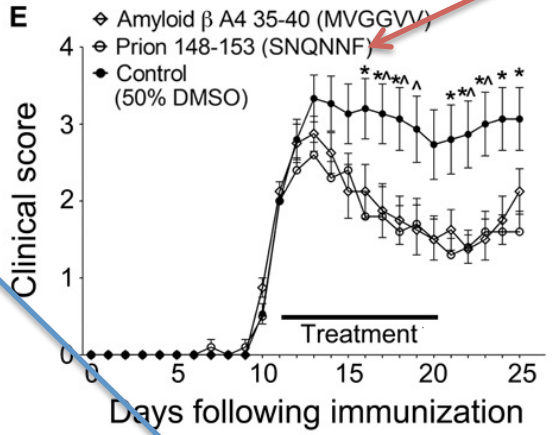
- Type I IFN, a common therapeutic for MS, has differential therapeutic activity in Th1 and Th17 EAE

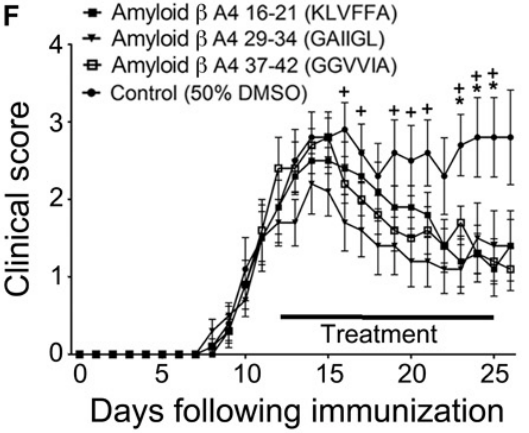


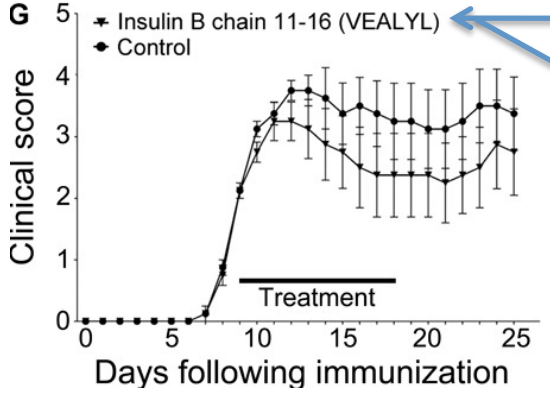






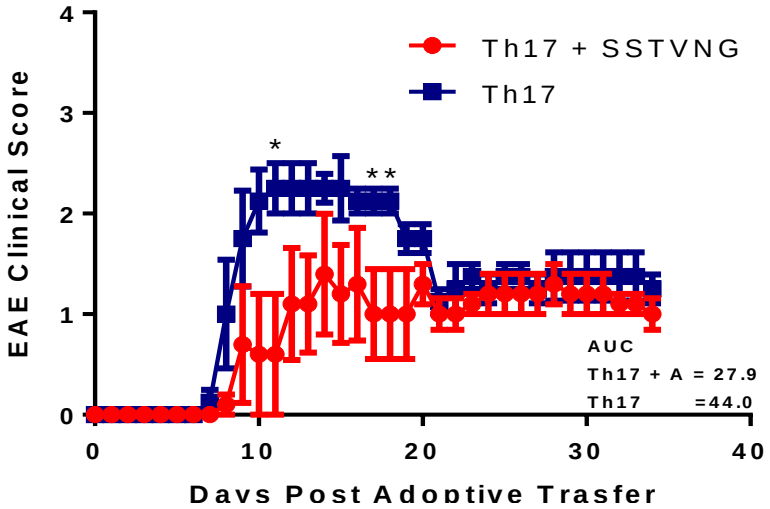


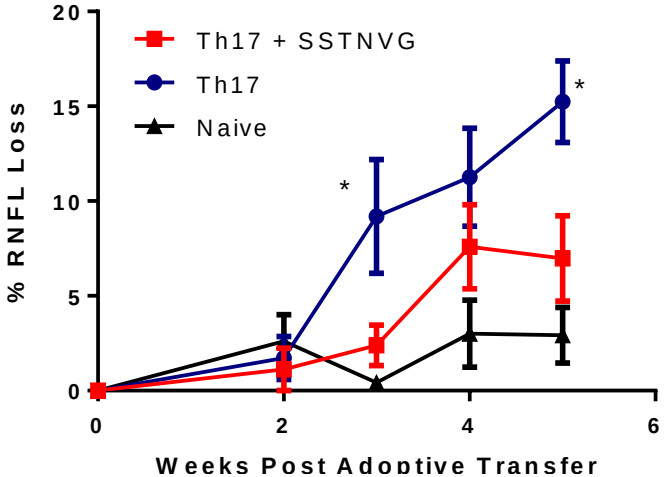




Two major secreted proteins from islets are amyloid:
Insulin !!! And Amylin!!! (Islet Associated Amyloid Protein
Is that Good or Bad Amyloid??

Hexapeptide Improve RNFL on OCT Dr. Kathryn Paunicka's Recent Work





Model of Th17 Optic Neuritis and Myelitis

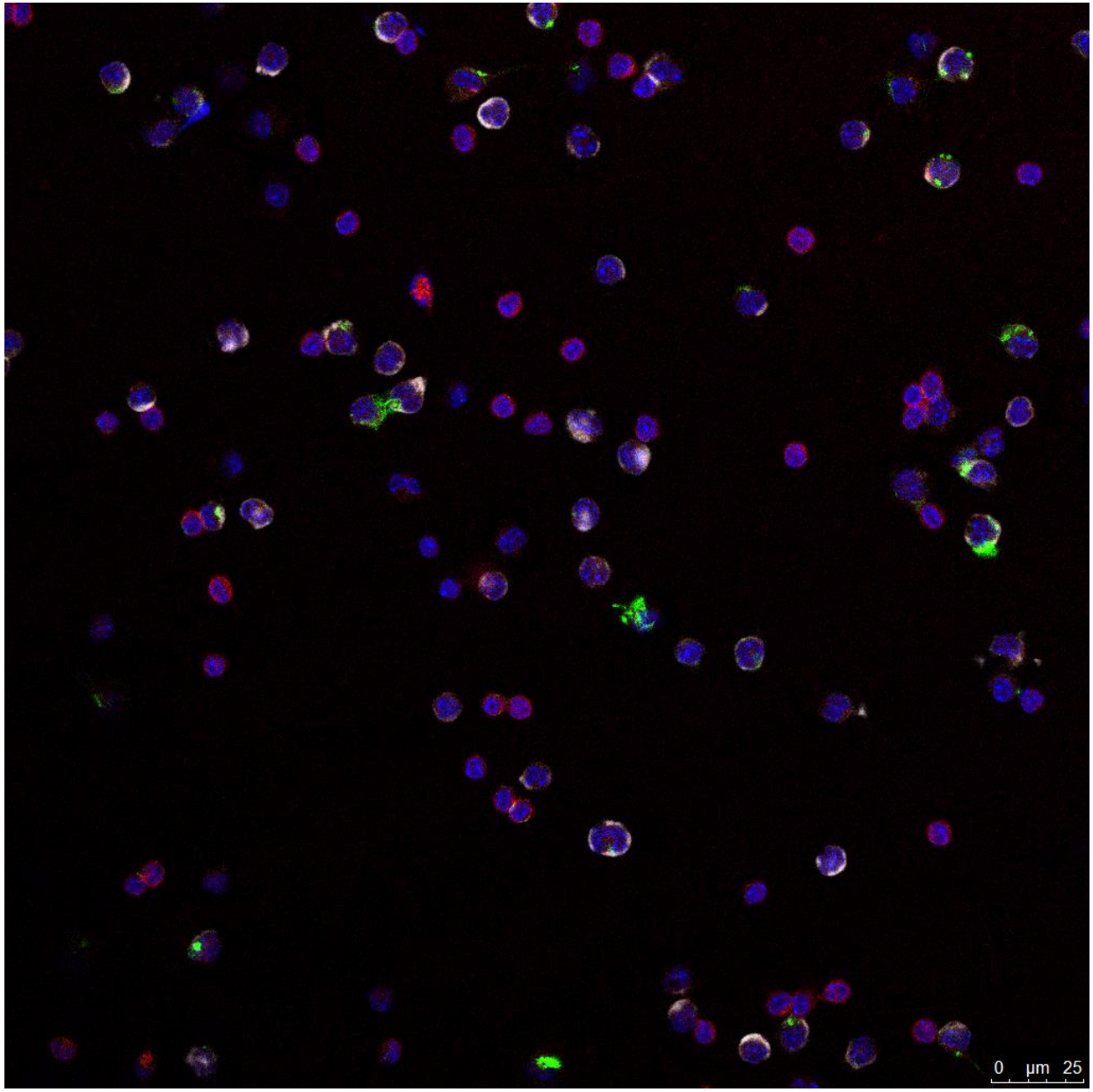
Mechanism 3 for Amyloid Hexapeptides

Amyloid Fibrils Activate B-1a Lymphocytes to Ameliorate Inflammatory Brain Disease

Michael P. Kurnellas[1], Eliver E. B. Ghosn[2], Jill M. Schartner[3], Jeanette Baker[4], Jesse J. Rothbard[1], Robert S. Negrin[4], Leonore A. Herzenberg[2], C. Garrison Fathman[3], Lawrence Steinman[1*], and Jonathan B. Rothbard[1,3]

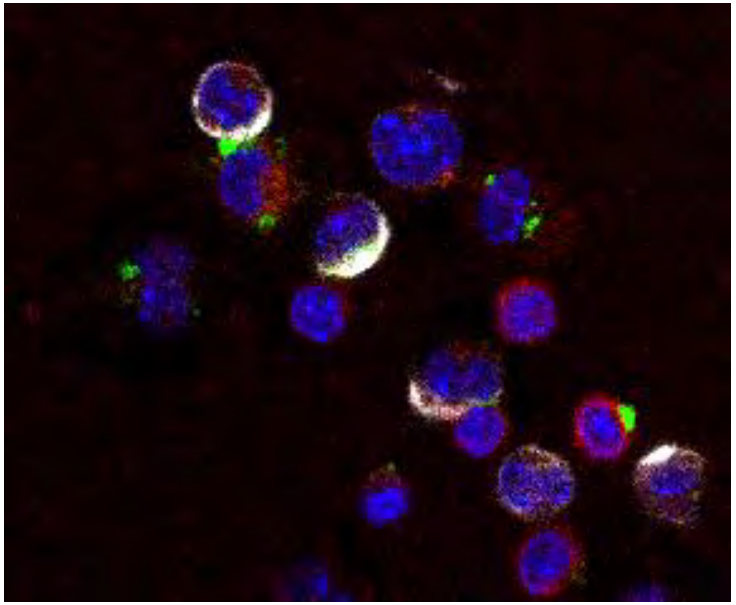
PNAS in press

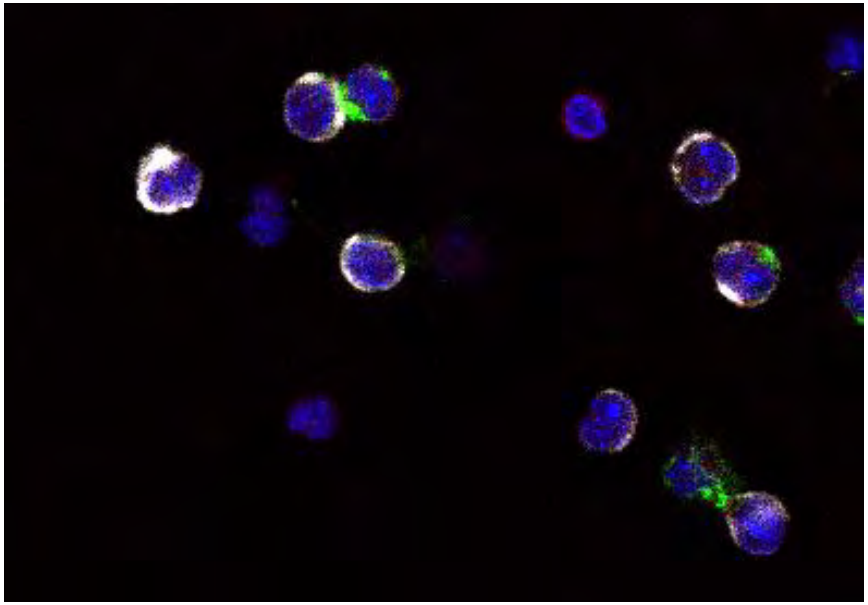
Amyloid fibrils associate with peritoneal B cells and MΦs

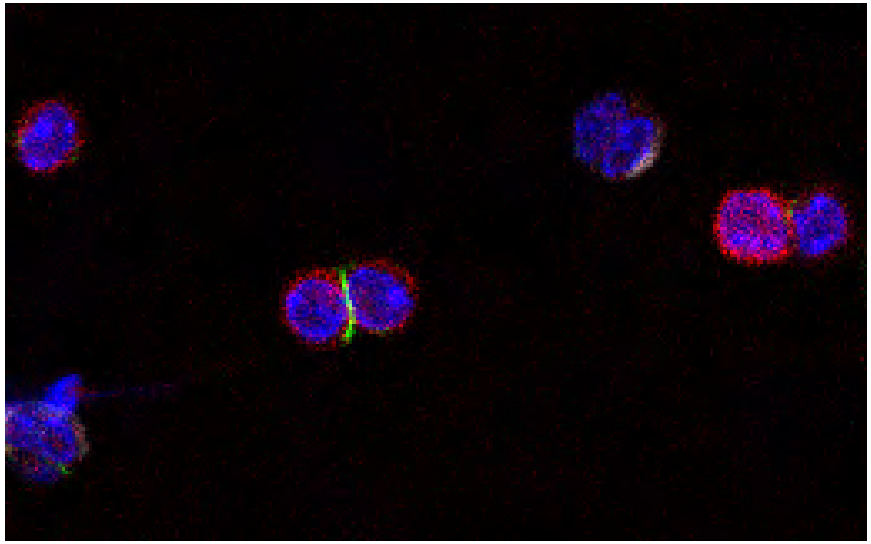


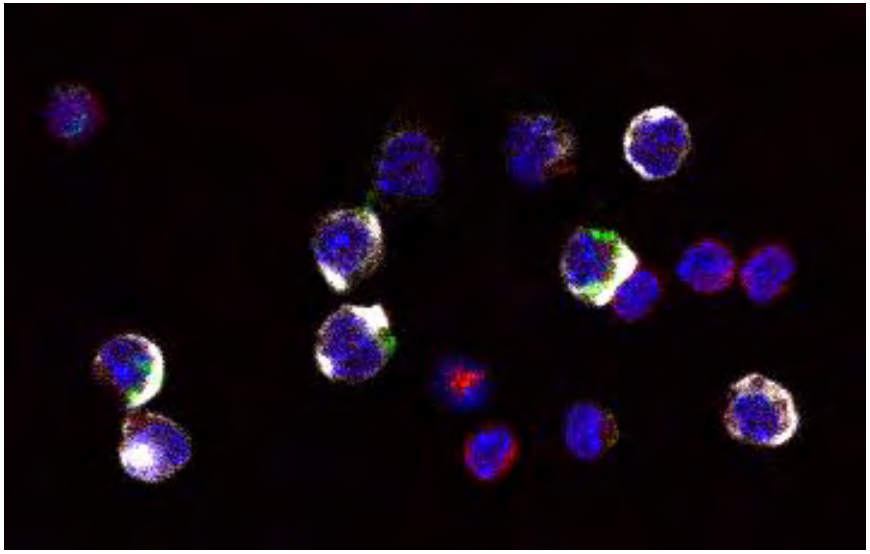
- FITC-Tau 623-628
- Macrophages
  - anti-F4/80
  - white
- B cells
  - anti-CD19
  - red

Peptide associated with macrophages (white) and B cells (red)

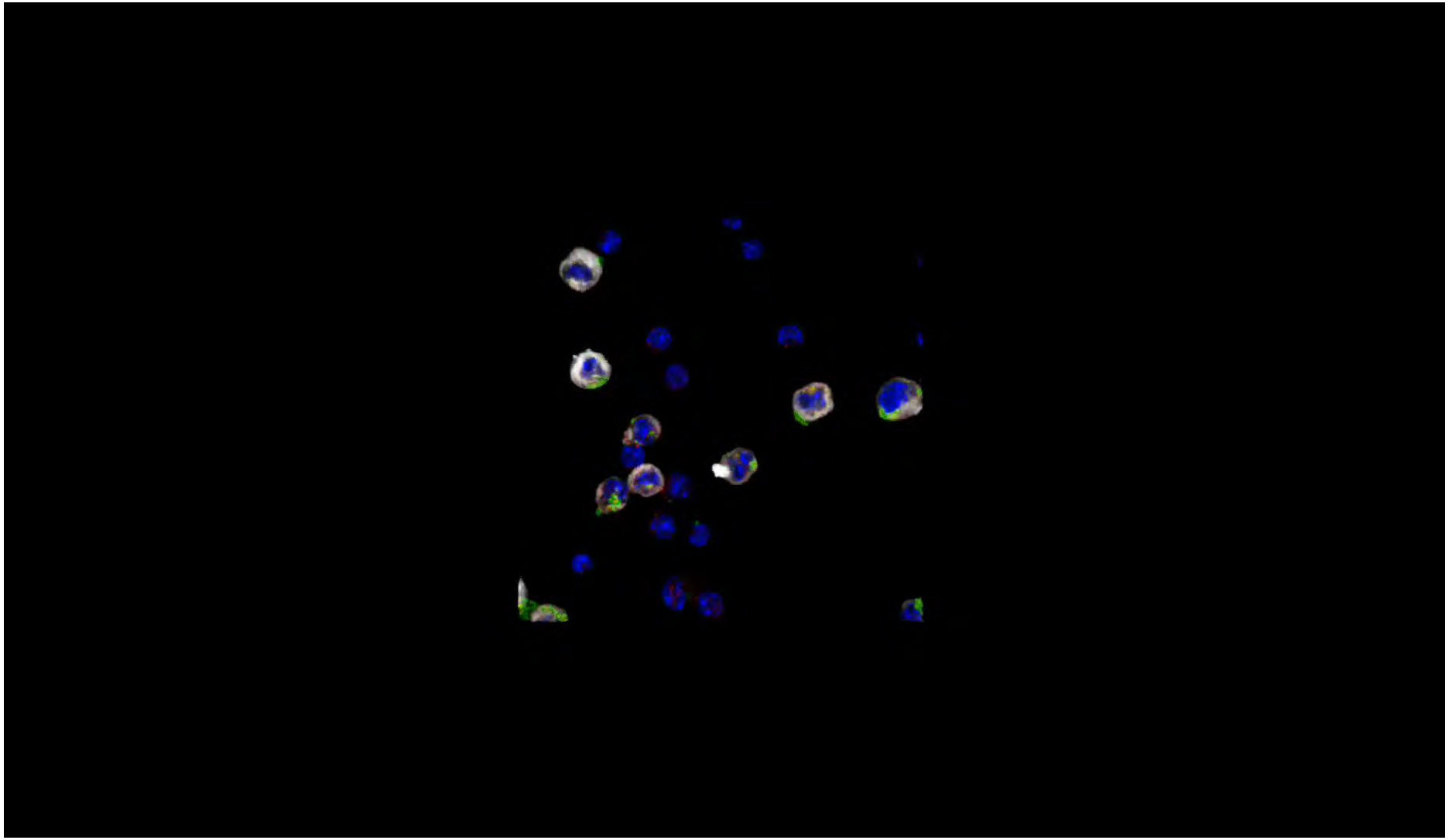




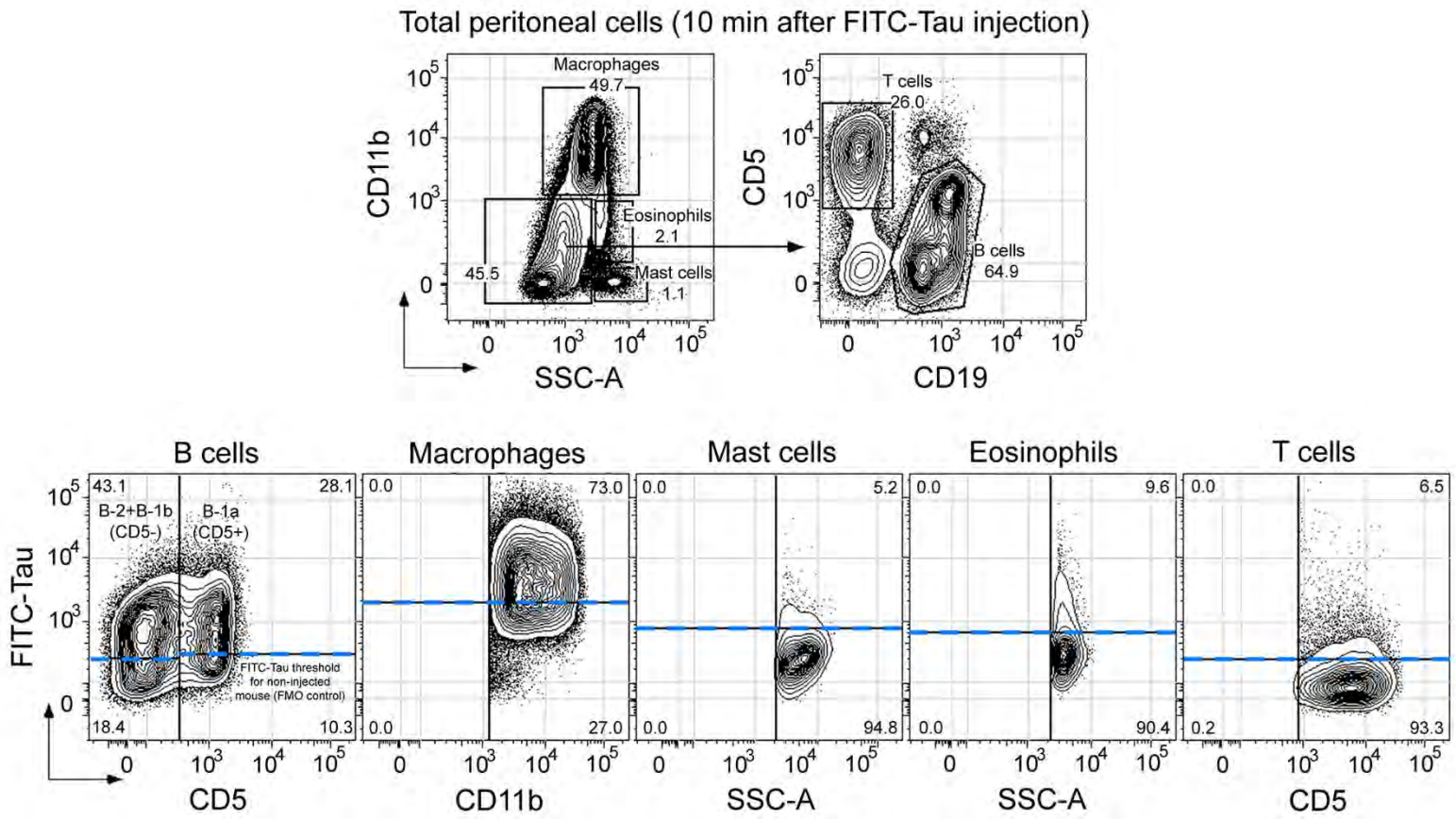




FITC-Tau 623-628 endocytosed by
B cells (red) and macrophages (white)

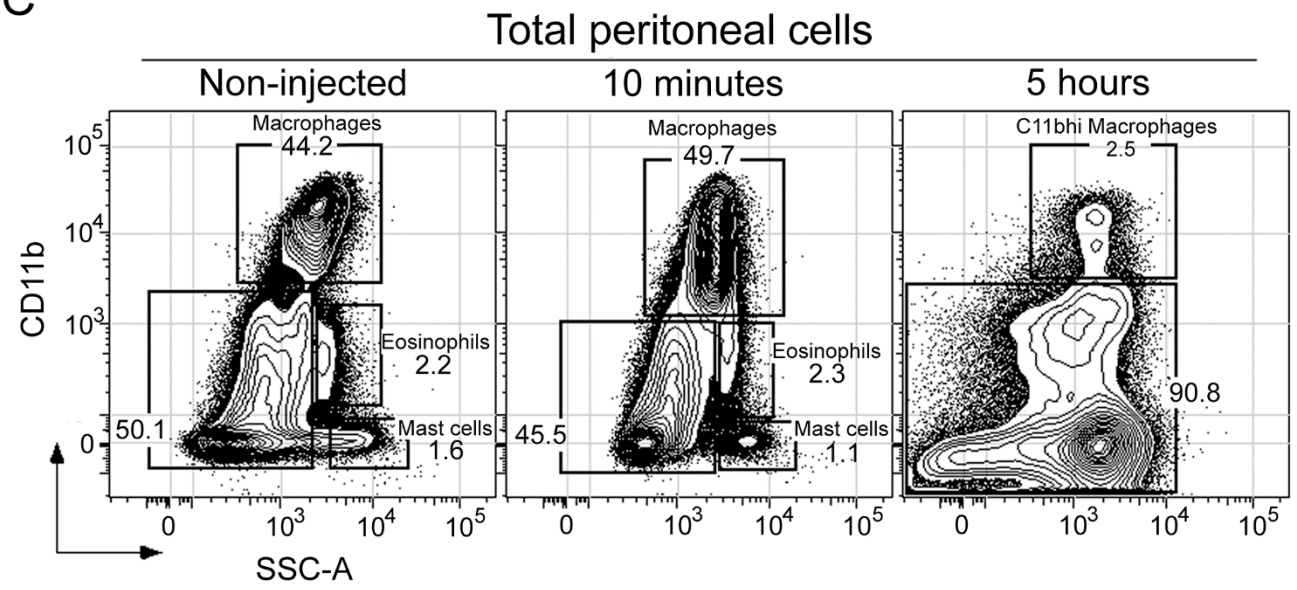


Flow cytometry confirmed and extended the microscopic study

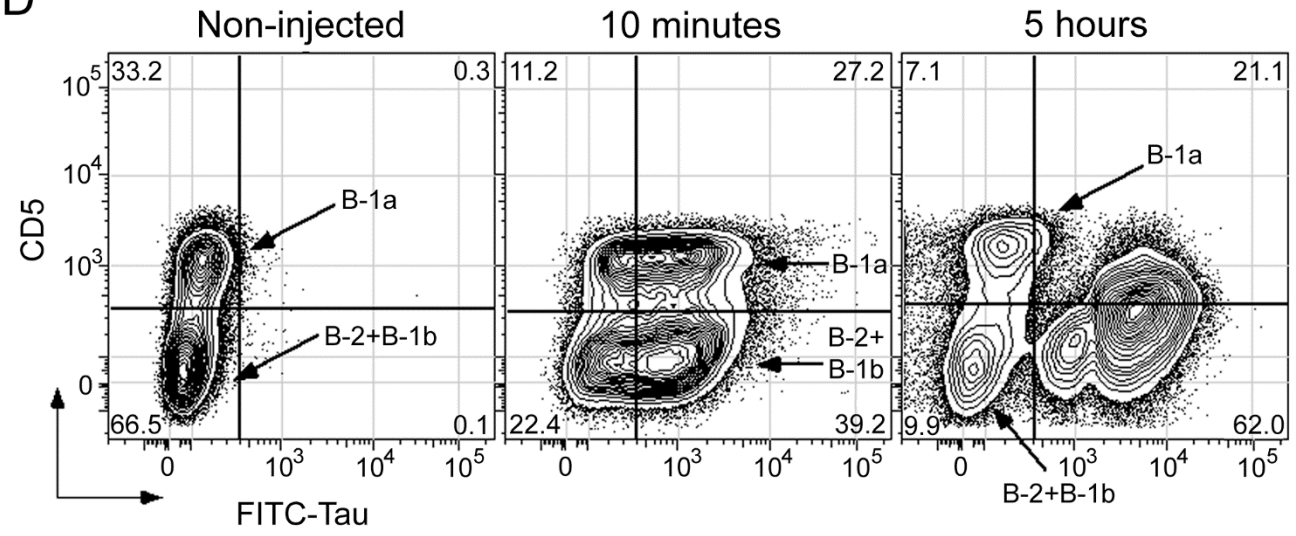


- Within 10 minutes of the FITC-Tau injection, more than 70% of the B-1 and B-2 lymphocytes and macrophages are FITC positive
- T lymphocytes and mast cells are minimally stained, demonstrating specific binding or uptake by B cells and MΦs

C



D



- After five hours, the majority of the CD11b high population is significantly reduced from approximately 45% to 3% of the total peritoneal cells
- Most of the B-1a population has disappeared, with the remaining cells being FITC-Tau negative

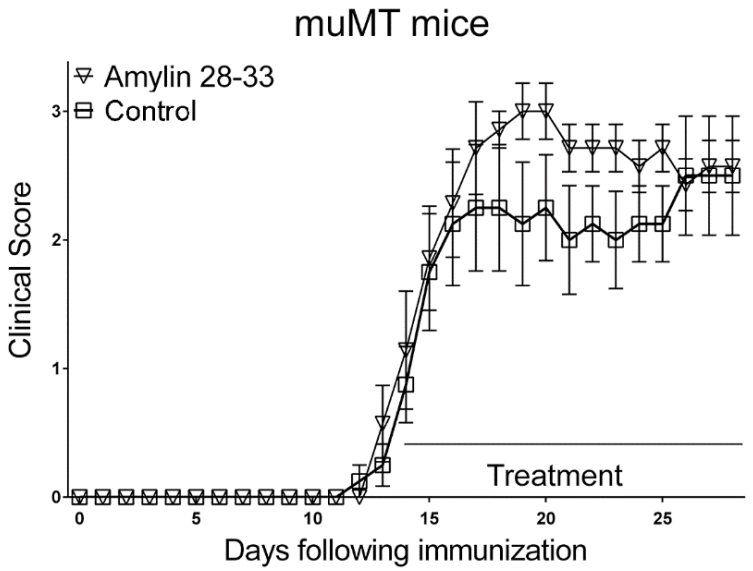
Use of μMT mice that lack mature B cells

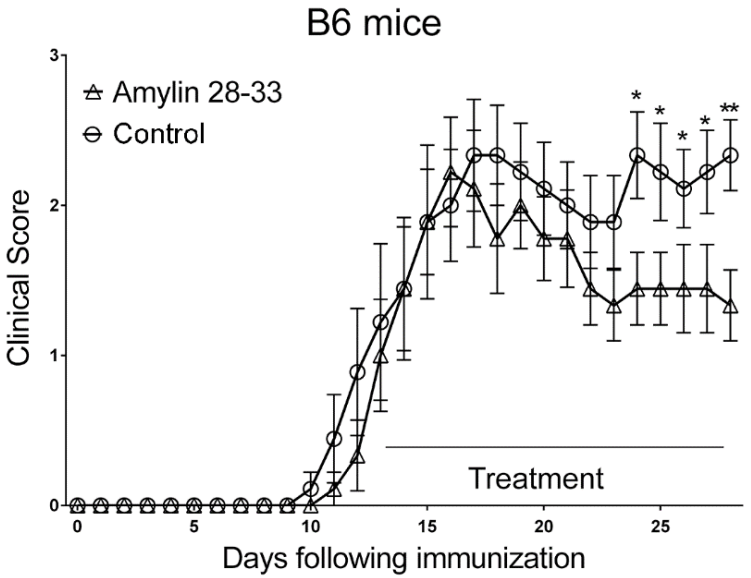
- A B cell-deficient mouse by targeted disruption of the membrane exon of the immunoglobulin μ chain gene. (Kitamura D., Roes J., Kuhn R., & Rajewsky K. Nature 1991; 350: 423-6).

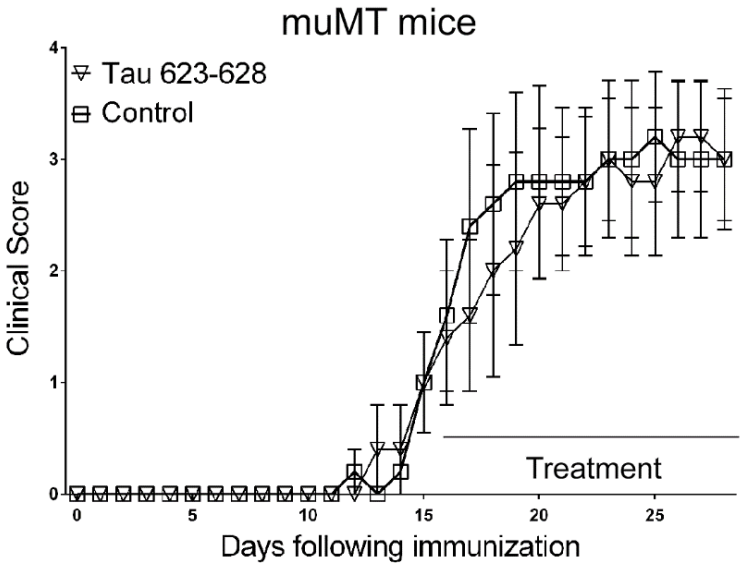
- B cell-deficient mice develop experimental allergic encephalomyelitis with demyelination after myelin oligodendrocyte glycoprotein sensitization. (Hjelmstrom P., Juedes A.E., Fjell J., & Ruddle N.H. J Immunol 1998; 161: 4480-3).

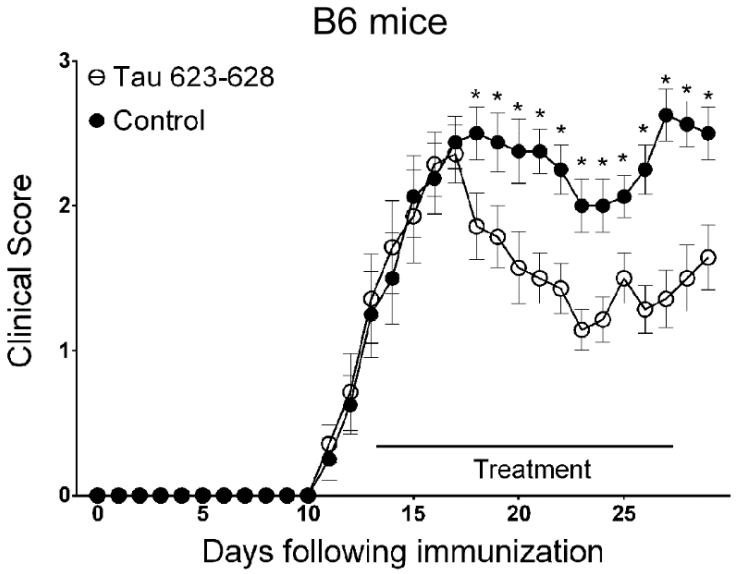
- Relapsing and remitting experimental autoimmune encephalomyelitis in B cell deficient mice. (Dittel B.N., Urbania T.H., Janeway, Jr. C.A. J Autoimmunity 2000; 14: 311-8).

Amyloid fibrils have no therapeutic efficacy in μMT mice





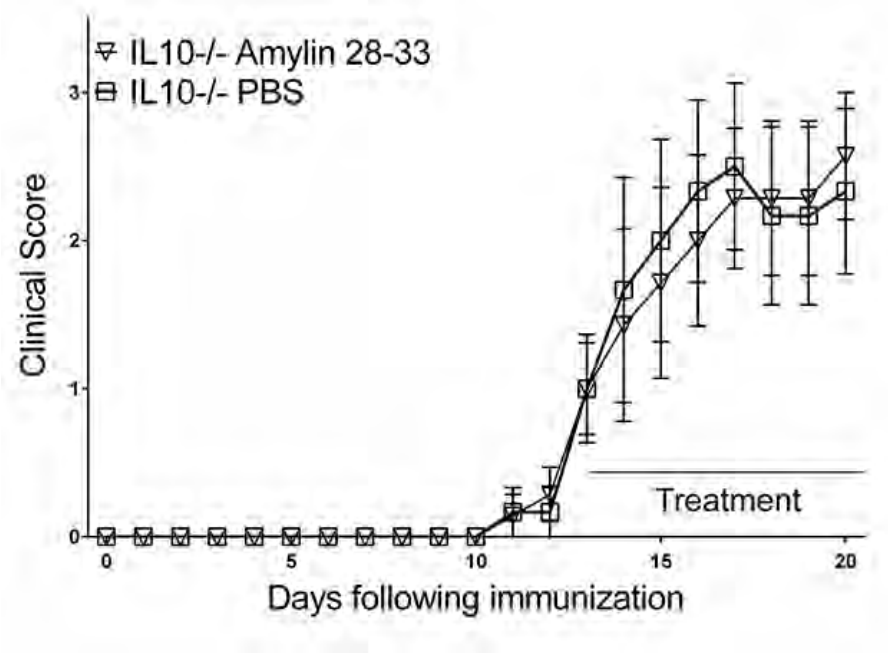


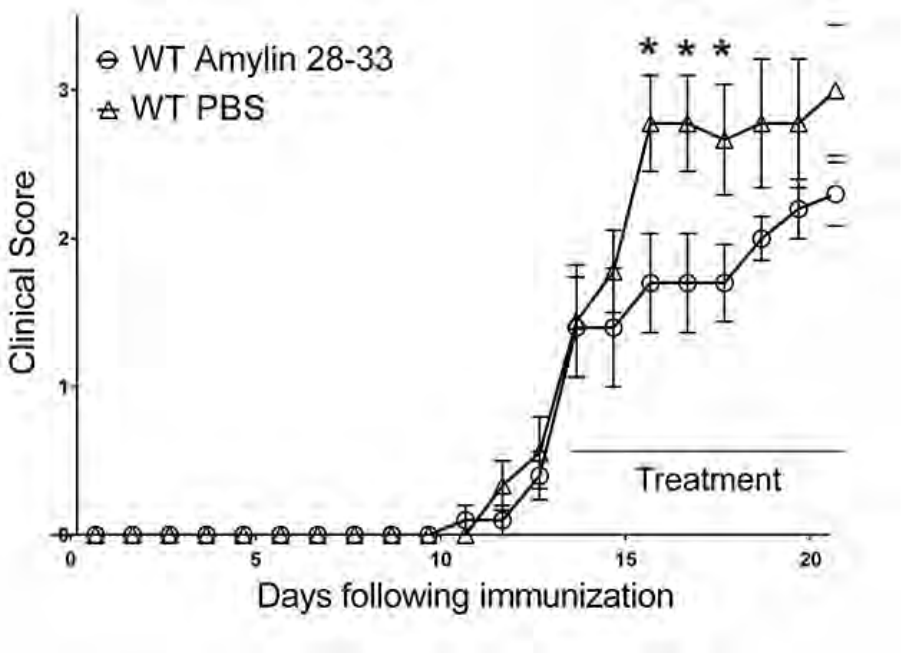


B cells are necessary for the therapeutic effect

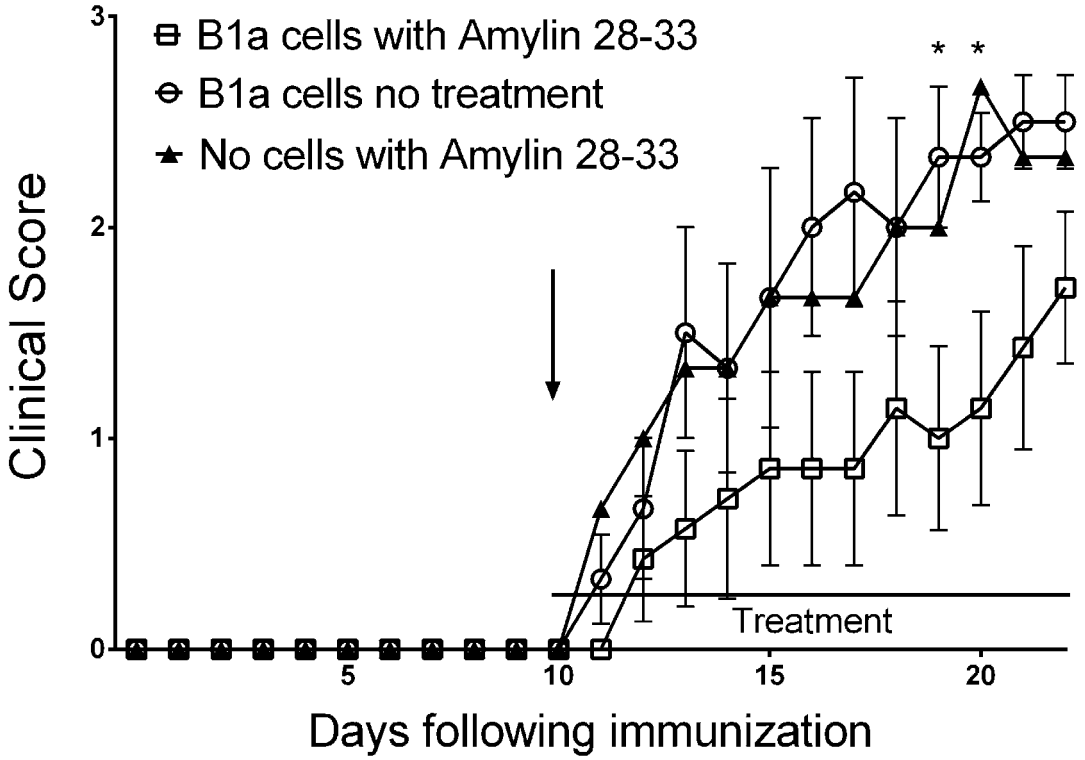
- B cells regulate autoimmunity by provision of IL-10 (Fillatreau et al., Nat Immunol 2002; 3: 944-50)
  - B cell deficient mice fail to resolve EAE
  - B cell IL-10 production required for recovery
  - Transfer of IL-10+ B cells suppresses EAE
- B-1a cells from peritoneal cavity are dominant producers of B cell-derived IL-10
- Next, we examined the role of IL-10 in the therapeutic efficacy of our peptides by using IL-10 deficient mice

Amylin 28-33 has no therapeutic efficacy in IL-10 deficient mice





Transfer of B1a cells into μMT mice treated with Amylin 28-33 restores therapeutic effect



- 3.5x10^5 B1a cells transferred into μMT mice on Day 10 post-immunization (arrow)
- B1a restores therapeutic efficacy of Amylin 28-33 in the μMT mice
- B1a cells alone cannot restore therapeutic effect

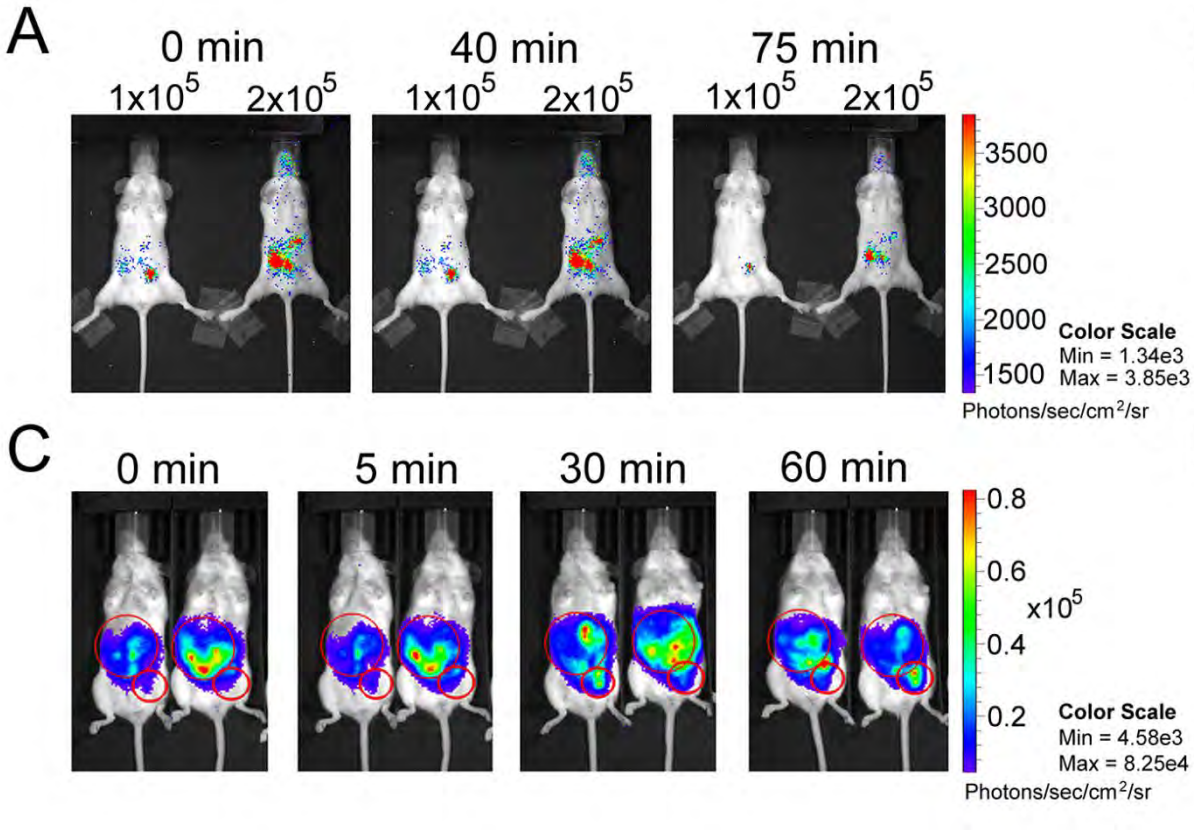
IL-10 vital for therapeutic efficacy

- B-1a cells from peritoneal cavity are dominant producers of B cell-derived IL-10

- B-1a cells migrate from PerC to spleen after LPS stimulation (Yang et al., PNAS 2007; 104: 4542-4546)

- **Hypothesis**: Amyloidogenic hexapeptides induce B-1a activation, resulting in migration to the lymph nodes, and suppression of inflammation through provision of IL-10

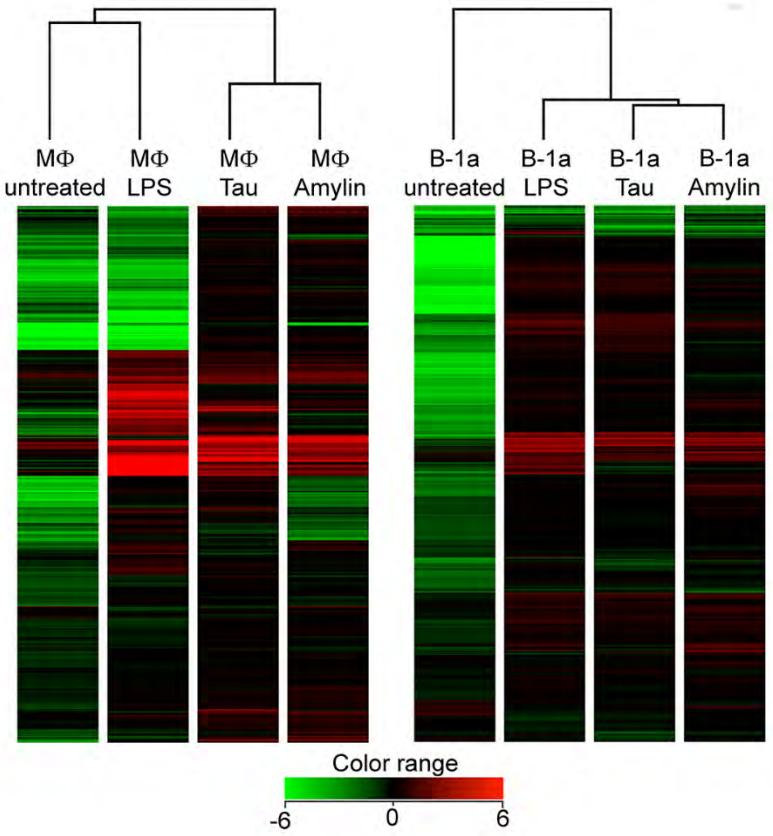
Amyloidogenic peptides induce exodus of B-1a cells and LPM from the peritoneal cavity



Utilization of gene microarray to identify targets

- B-1a cells and macrophages endocytose the self-assembling peptides
- Activation of these cell types occur
- Use of gene microarray
  - Wild type mice treated for 40 minutes
    - Lactated Ringer's solution
    - Amylin 28-33, 10μg
    - Tau 623-628, 10μg
    - LPS, 10μg
  - Peritoneal cavity cells isolated by flow
    - Isolated B-1a cells
    - Isolated macrophages
  - Collect RNA and run microarray

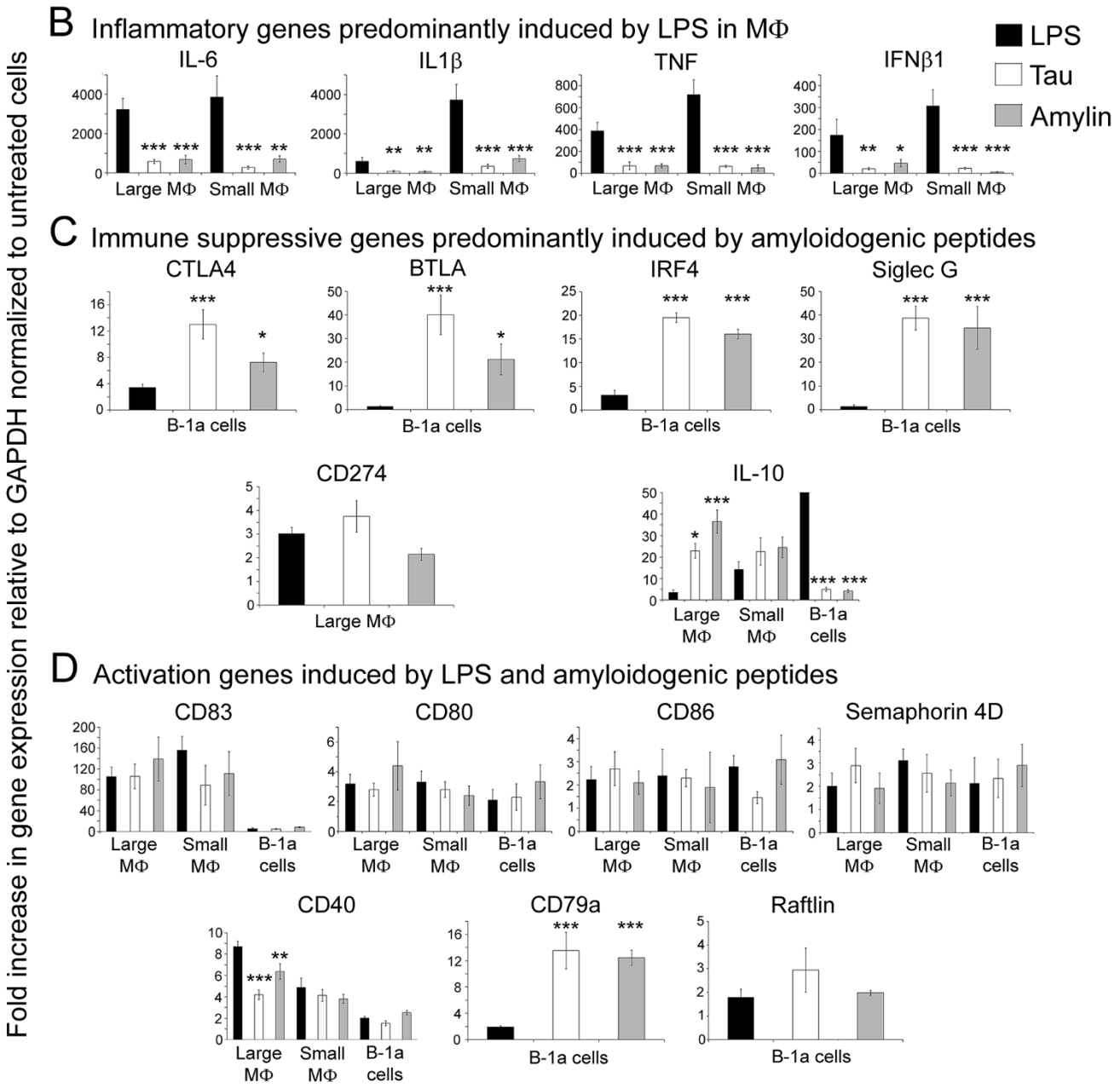
Amyloid fibrils induce a different pattern of gene expression than LPS in B-1a lymphocytes and peritoneal MΦs



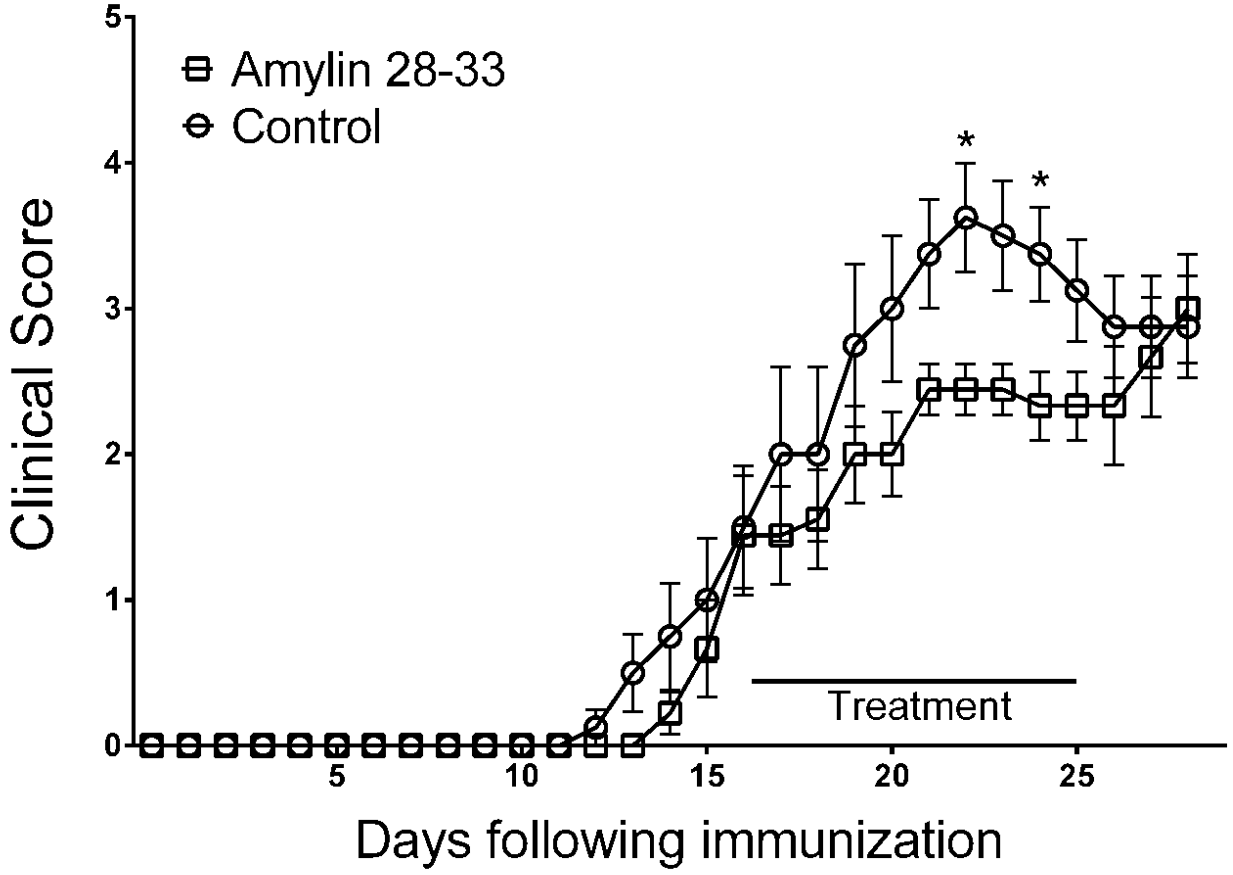
- Differential gene expression (720 annotated genes) expressed as a heatmap induced by LPS and the two types of amyloid fibrils, Tau 623-628 and Amylin 28-33

- RNA isolated from purified B-1a lymphocytes and CD11b^{high} MΦs isolated from groups of three C57BL/6 mice injected with either 10μg LPS, Amylin 28-33, Tau 623-628, or buffer.

Measurement by qPCR sets of genes representing (B) inflammatory cytokines, (C) immune suppressive genes, or (D) activation genes.



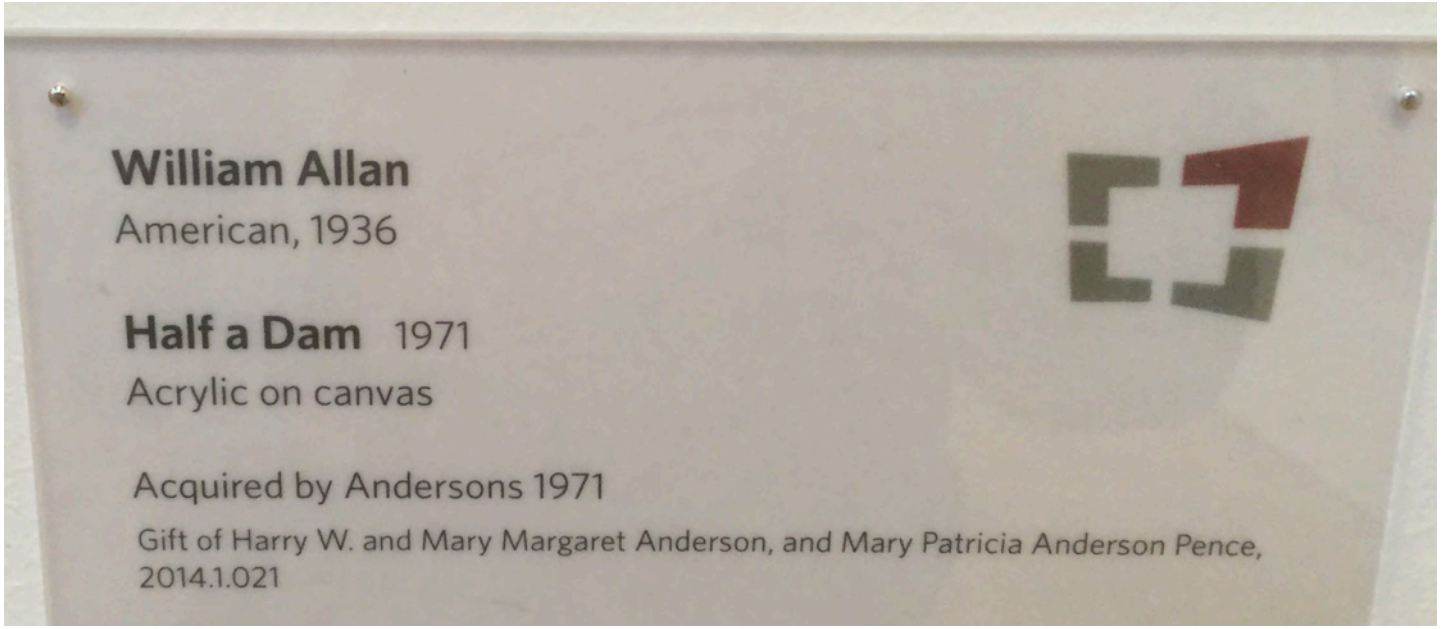
B-1a cells are present in the lung. Is Amylin 28-33 effective by intranasal administration?



Summary of mechanism of action

1) Fibrils can activate B-1a cells, which can provide local delivery of IL-10
2) IL-10 inhibits both APC and T cell-based inflammation
3) There are no known agents capable of selectively activating regulatory B cells
4) The effect occurs in the lymph node, not at sites of inflammation in the CNS, no need to cross BBB
5) MOA predicts that fibrils should be effective in large numbers of diseases

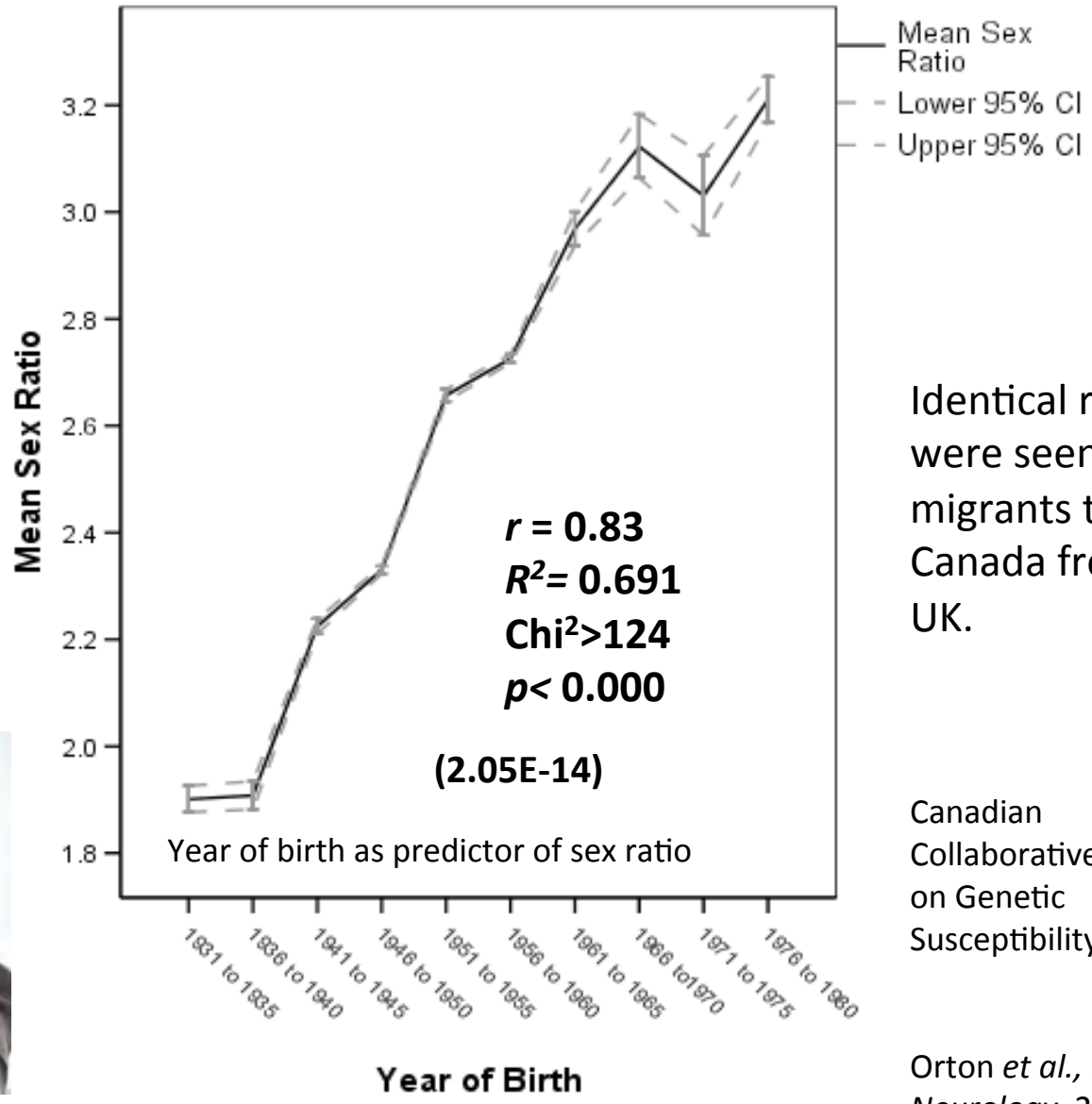
California Funk and Amyloid


William Allan
American, 1936
Half a Dam 1971
Acrylic on canvas
Acquired by Andersons 1971
Gift of Harry W. and Mary Margaret Anderson, and Mary Patricia Anderson Pence,
2014.1.021

In the next 50 minutes I shall share with you some "tractable" targets derived from various "omics", without the aid of "genomics". I shall describe the following:

1. The inhibitory neurotransmitter GABA is immune suppressive
2. Angiotensin Receptors are in MS Lesions
   ACE inhibition is beneficial in animal models
3. Immune suppressive lipids in the myelin sheath
4. Infamous amyloid proteins provide protection, not harm in neuroinflammatory conditions
5. **PPARs are targetable natural "brakes" on neuroinflammation. They may also help explain gender disparity in MS**

Sex Ratio (F:M) by year of birth the past half-century



Identical results were seen in migrants to Canada from the UK.

Canadian Collaborative Project on Genetic Susceptibility

Orton *et al., Lancet Neurology,* 2006

Nuclear Hormone Receptors
Top Down Discovery: Implications for MS

RESEARCH HIGHLIGHTS

NEUROBIOLOGY

Prion symptoms reversed

Neuron **53**, 325–335 (2007)

Early symptoms of the neurodegeneration caused by prion disease can be reversed in genetically engineered mice, report Giovanna Mallucci, of the Medical Research Council's Institute of Neurology in London, and her colleagues.

The researchers monitored the behaviour of mice infected with a prion protein to look for early indicators of the disease. Changes in the way the mice responded to their environment occurred before the onset of obvious signs of neurodegeneration, such as reduced grooming. The mice recovered their normal brain function if production of the naturally occurring protein that propagates the disease was switched off at this stage.

But the implications for treatment of the human prion disease, Creutzfeldt–Jakob disease, are uncertain. It's not clear whether [illegible]s could recover, nor how levels of the [illegible]tein could be lowered.

IMMUNOLOGY

The right kind of help

J. Exp. Med. doi:10.1084/jem.20061839 (2007)

So far they have only tested their hypothesis in mice, but researchers think they have identified a mechanism that could help to explain why men are less prone than women to developing certain types of autoimmune disease, such as mutliple sclerosis.

Lawrence Steinman of Stanford University Medical Center, California, and his colleagues studied a receptor known as peroxisome proliferator-activated receptor-α, which has been implicated in gender differences in lipid metabolism. The receptor is also expressed in the immune system's $CD4^+$ T cells.

The researchers showed that the receptor gene is sensitive to testosterone, and is expressed at higher levels in the T cells of male mice than in those of females.

$CD4^+$ T cells differentiate into different types of 'T-helper' cell. Expression of the receptor seems to direct differentiation away from the type that is associated with certain autoimmune diseases. Knocking out the gene in males made the symptoms of a mouse model of multiple sclerosis more severe.

PALAEONTOLOGY

Could the 'hobbit' hunt?

HOMO — J. Comp. Hum. Biol. doi:10.1016/j.jchb.2006.11.001 (2007)

Debate over the diminutive *Homo floresiensis* — believed to be a hobbit-sized species of hominid — has inspired a team at Washington University in St Louis, Missouri, to develop a method to estimate the size of hominid brain components from fossil skulls.

Researchers have questioned whether the small-brained *H. floresiensis*, which lived on an isolated Indonesian island until at least 12,000 years ago, would have been capable of creating tools, using fire and hunting, as some studies have suggested.

Glenn Conroy and Richard Smith looked at the volumes of 11 different brain components in 45 primate species to set limits on the size of each component as a fraction of overall brain size. The predicted bounds for the brain of *H. floresiensis* are not

Published February 19, 2007
JEM ARTICLE

Peroxisome proliferator–activated receptor (PPAR)α expression in T cells mediates gender differences in development of T cell–mediated autoimmunity

Shannon E. Dunn,[1] Shalina S. Ousman,[1] Raymond A. Sobel,[2] Luis Zuniga,[1] Sergio E. Baranzini,[3] Sawsan Youssef,[1] Andrea Crowell,[1] John Loh,[1] Jorge Oksenberg,[3] and Lawrence Steinman[1]

Published July 12, 2010
JEM Brief Definitive Report

Peroxisome proliferator–activated receptor δ limits the expansion of pathogenic Th cells during central nervous system autoimmunity

Shannon E. Dunn,[1,6] Roopa Bhat,[1] Daniel S. Straus,[5] Raymond A. Sobel,[2] Robert Axtell,[1] Amanda Johnson,[1] Kim Nguyen,[1] Lata Mukundan,[3] Marina Moshkova,[6] Jason C. Dugas,[4] Ajay Chawla,[3] and Lawrence Steinman[1]

Nature Medicine **15**, 1266 - 1272 (2009)
Published online: 18 October 2009 | doi:10.1038/n

PPAR-δ senses and orchestrates clearance of apoptotic cells to promote tolerance

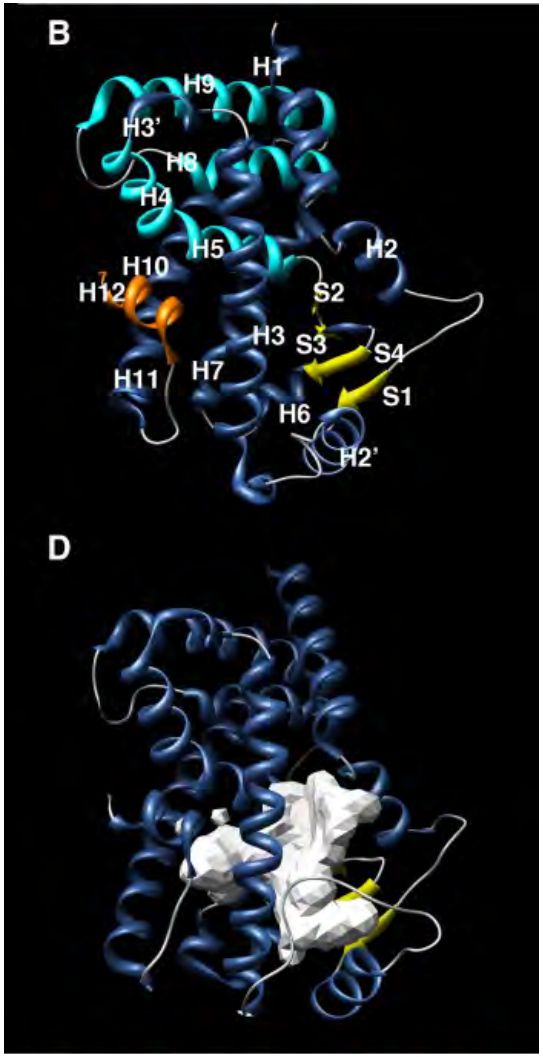
Peroxisome Proliferator-Activated Receptors (PPARs)

transcription factors- nuclear hormone receptor family

three family members (PPAR-α, -β/δ, -γ)

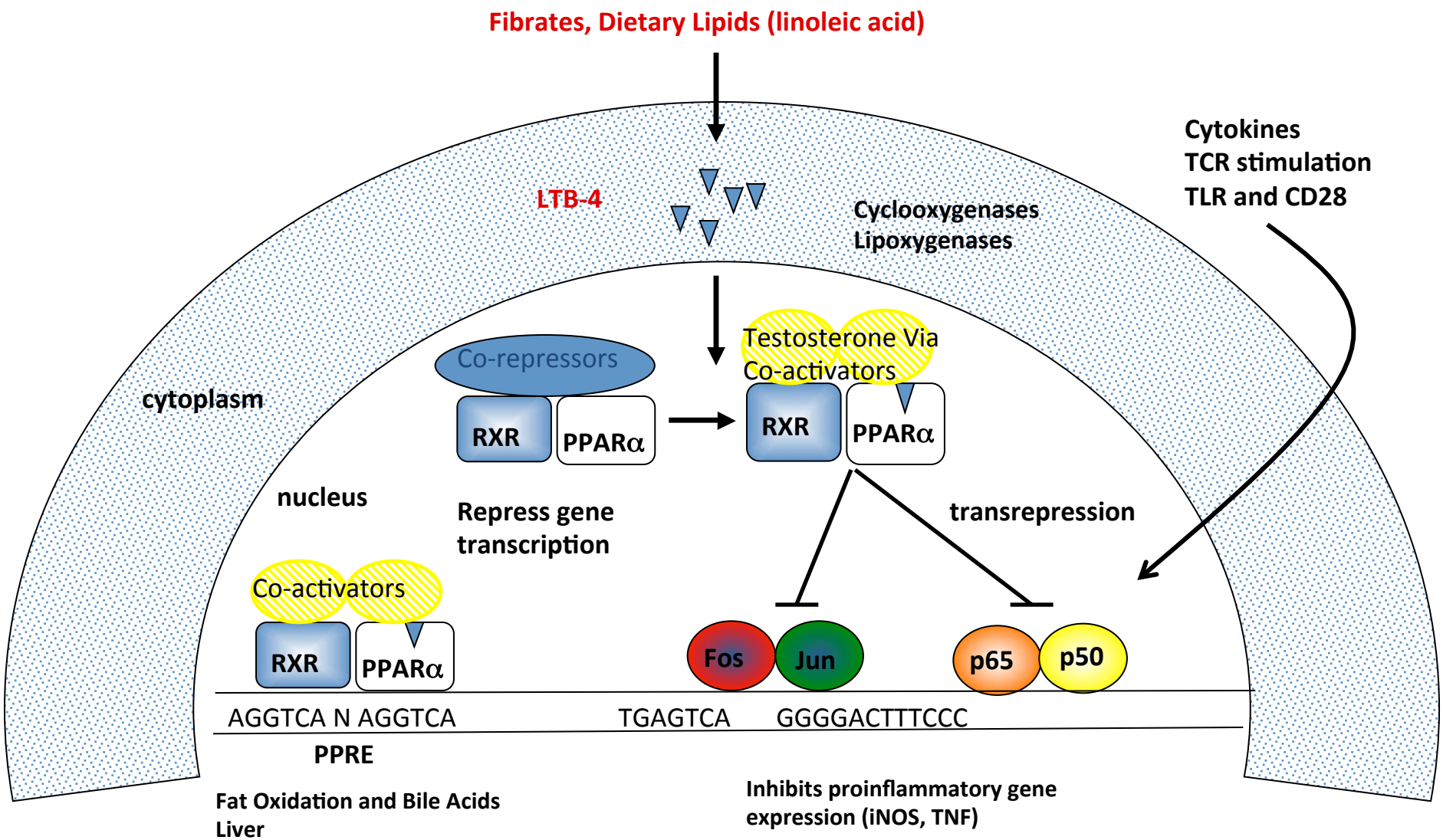
bind endogenous fatty acids (µM).

Drugs for T2DM and Hyperlipidemia

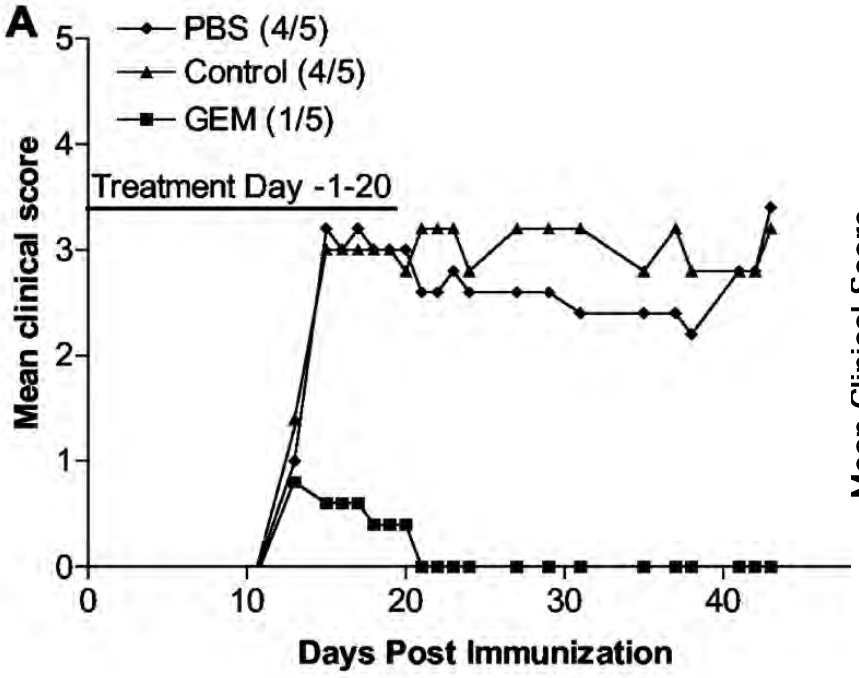


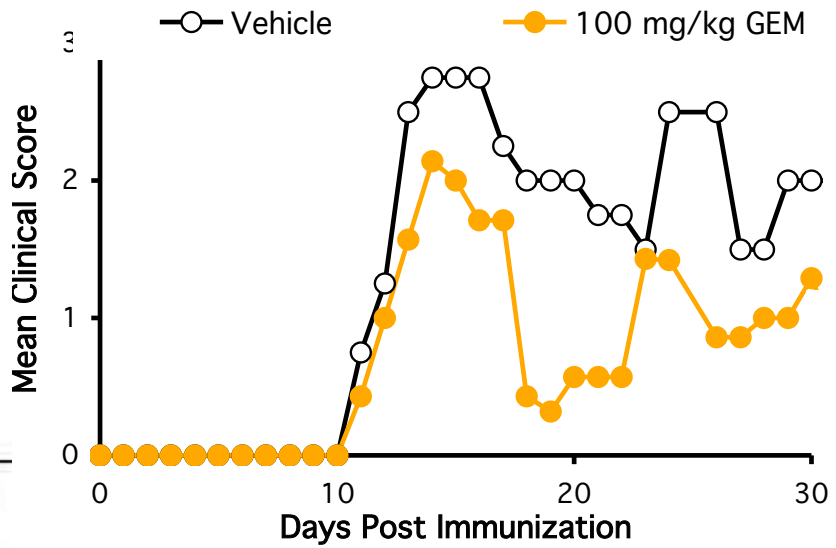
Zoete, *Biochim. Biophys. Acta* 1771, 915, 2007

PPARα and other PPARs are Anti-Inflammatory



PPARα Agonist Gemfibrozil (Lopid®) Ameliorates EAE

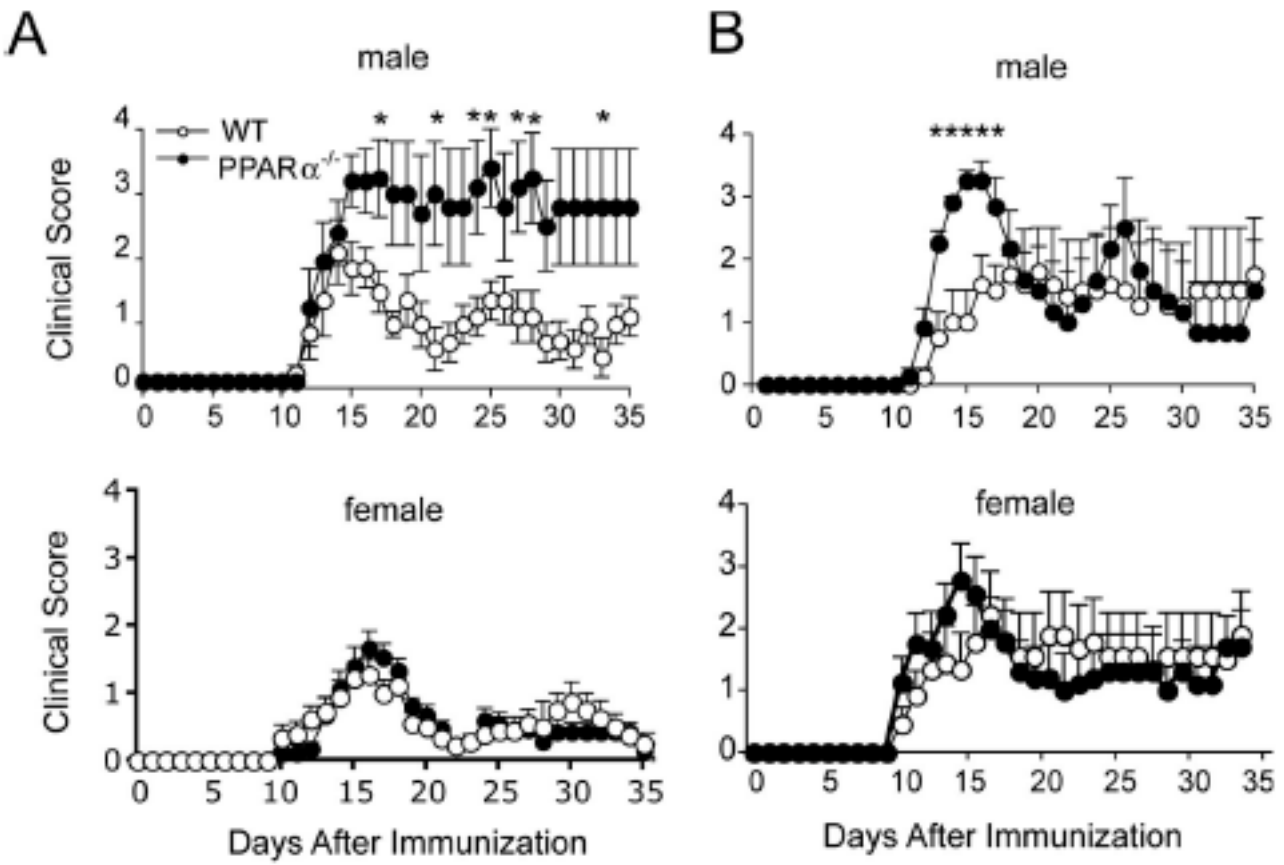




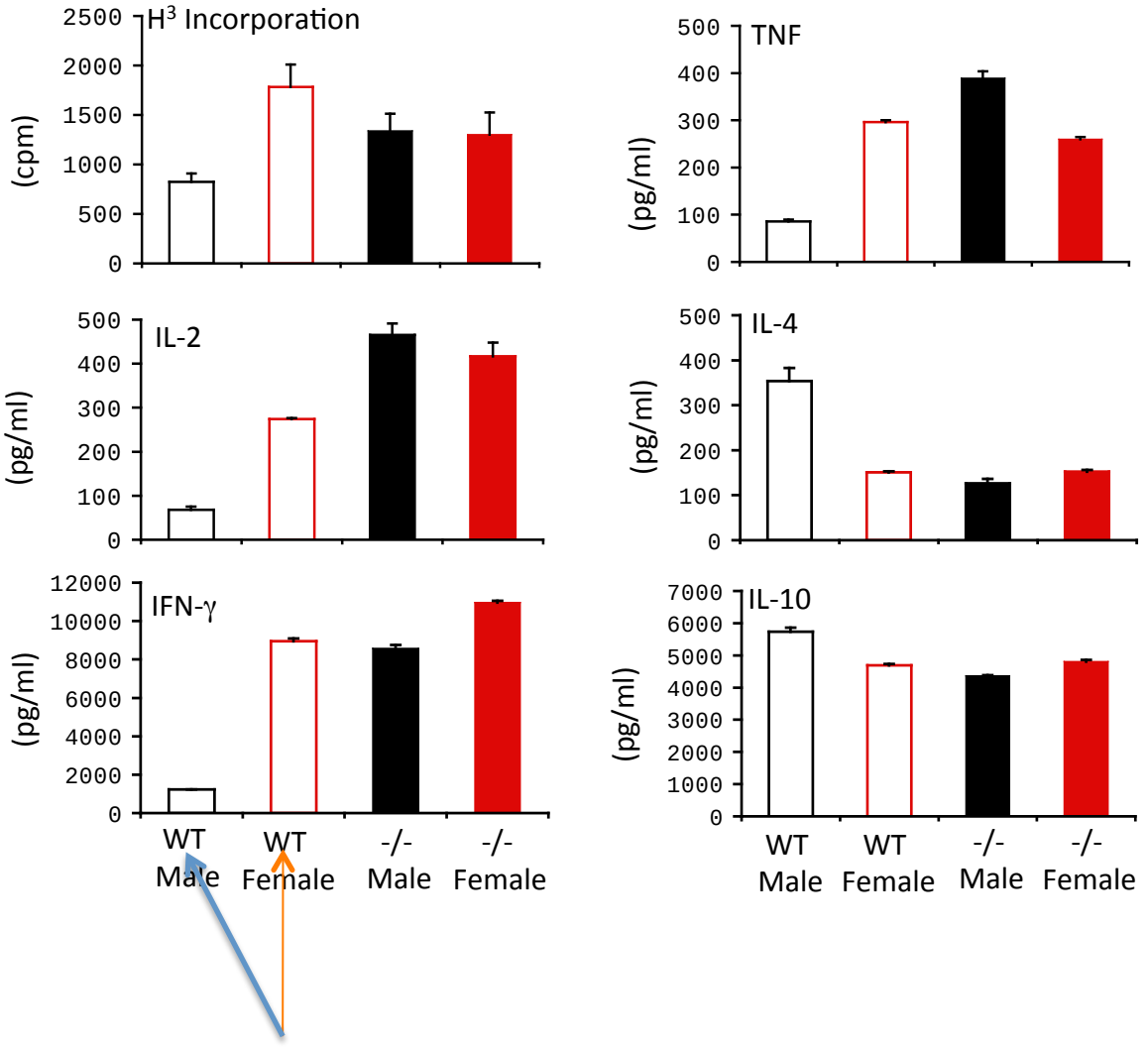
Lovett-Racke *et al., J. Immunol.* 172: 5790-5798, 2004.

Dunn, J Exp Med 2007

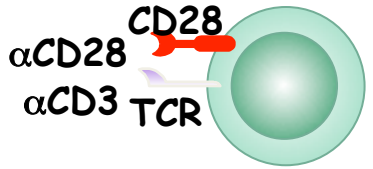
Male PPARα$^{-/-}$ Mice Develop Worse Acute EAE



Deficiency of PPARα in T cells: male v female
Female Mice Stronger Th1, until PPARα knocked out

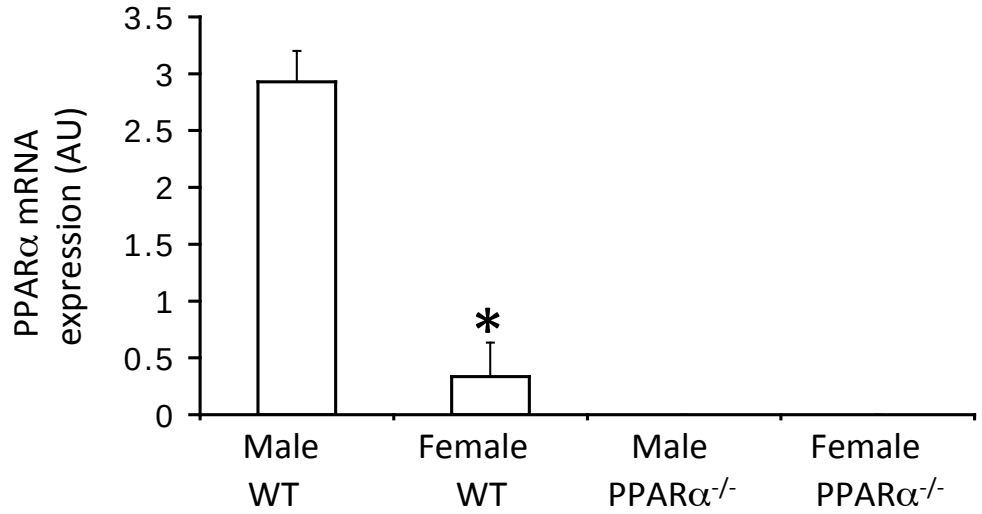


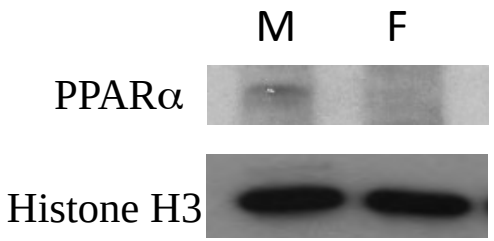
- Male WT
- Male PPARα$^{-/-}$
- Female WT
- Female PPARα$^{-/-}$



(0.5 μg/ml)

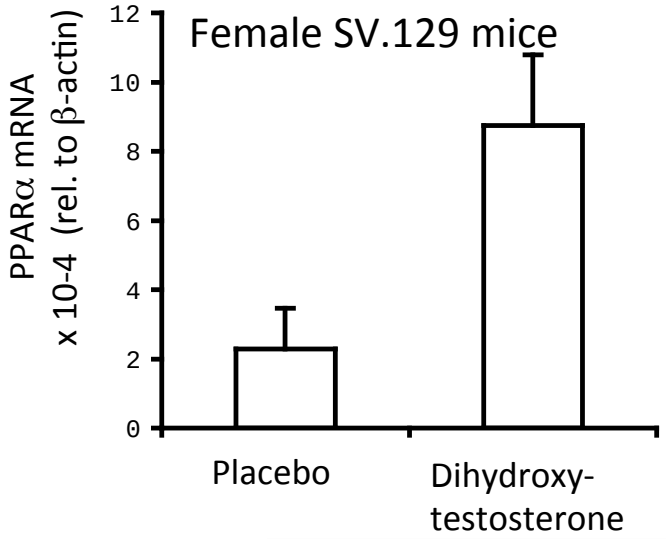
PPARα expression is higher in male CD4$^+$ T cells.





Dunn, S. E. et al. *J. Exp. Med*. **204:321-330, 2007.**

Surgical castration decreases PPARα
dihydroytestosterone treatment increases PPARα



Male
WT

Surgical Castration
Sham Surgery

Female
WT

5α-dihydrotestosterone (60 d release, 5 mg)

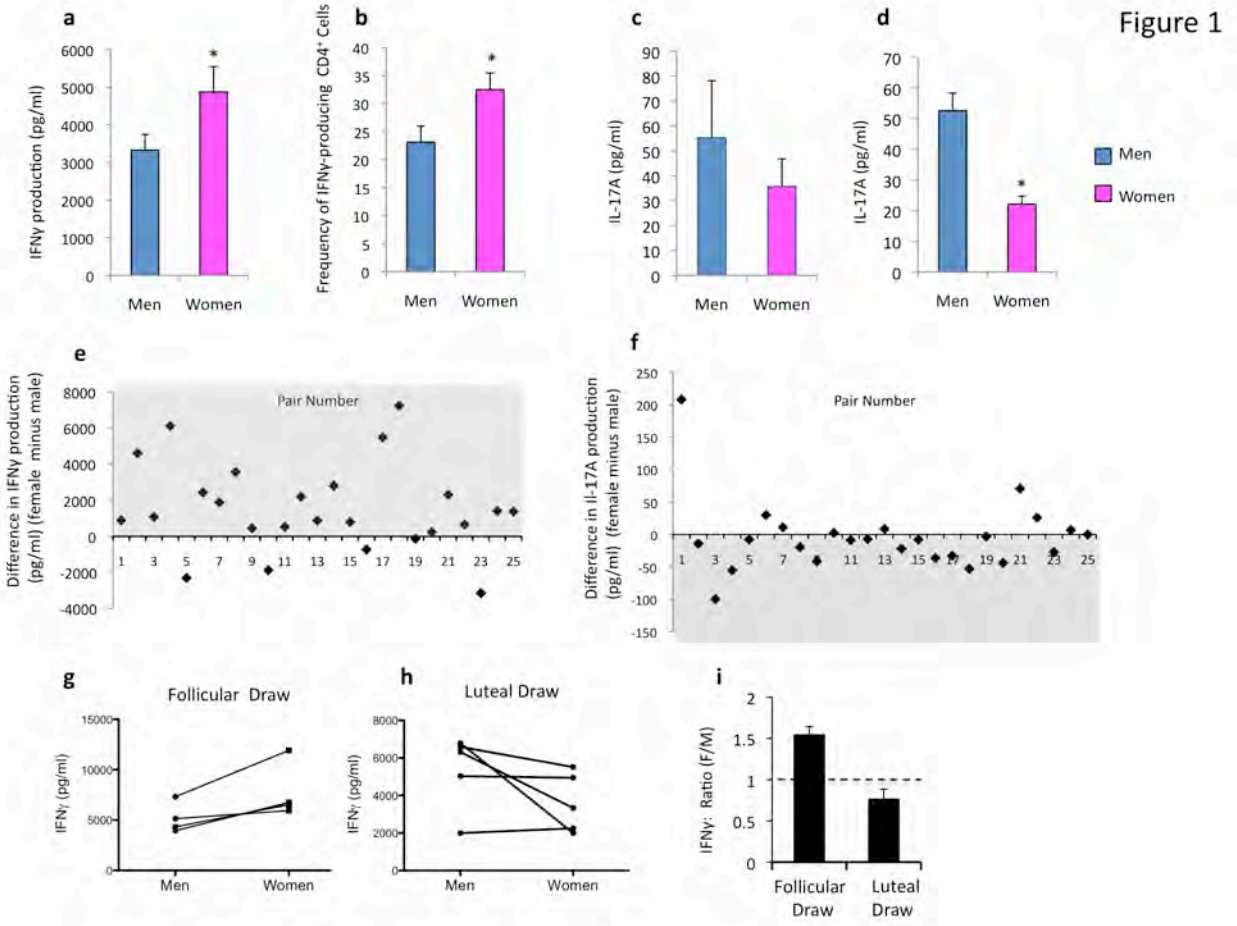
Placebo

Dunn, S. E. et al. *J. Exp. Med*. **204:321-330, 2007.**

HUMANS!

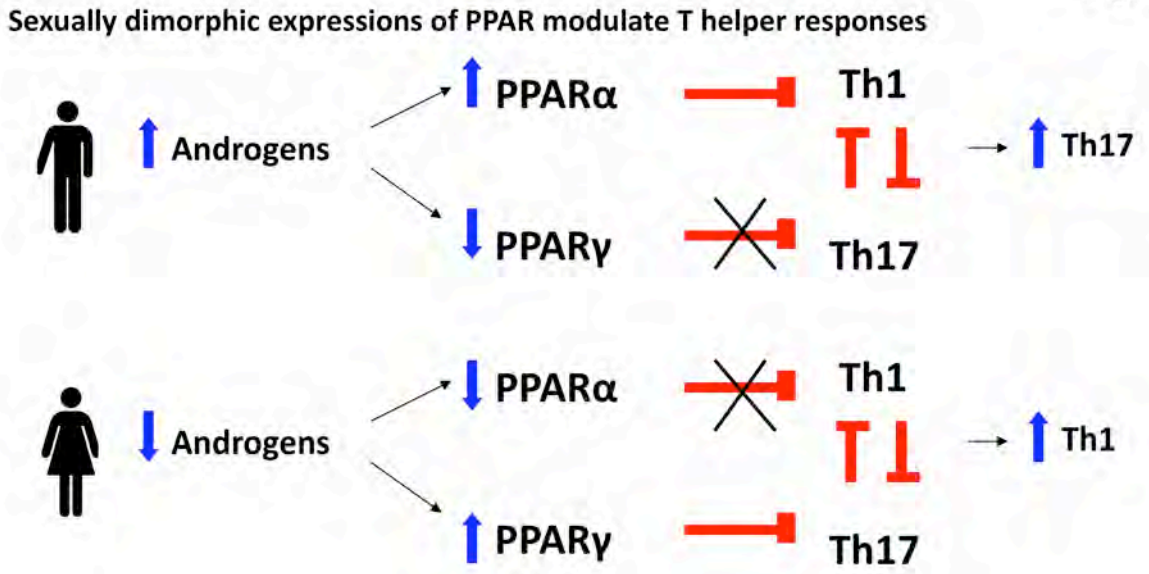
Females Produce More Th1, Males More Th17

Androgens Enhance PPAR-α and Inhibit PPAR-γ



Human and Mouse Females Make More IFN-γ

Fig. S4



Legend. Models of Sexual Dimorphism in Th Cytokine Production. A higher androgen level in men enhances PPARα expression, yet decreases PPARγ expression in activated naïve $CD4^+$ T cells. As a result, the brake normally imposed on Th17 by PPARγ is removed while PPARα continues to inhibit Th1. Consequently, male T cells are more Th17-prone. Conversely, a lower androgen level in women leads to decreased PPARα expression yet enhanced PPARγ expression in naïve $CD4^+$ T cells upon stimulation. As a result, the break on Th1 by PPARα is lifted while the Th17 suppression by PPARγ persists. Consequently, we found female T cells to be more Th1-prone.

Does this explain the rising incidence of MS in Females?

Not exactly, but Dunn's discoveries illuminate a 'stunning' dimorphic brake on immunity

Dunn's discoveries open the possibility of re-purposing drugs targeting PPAR's to treat MS

In the next 50 minutes I shall share with you some “tractable” targets derived from various “omics”, without the aid of “genomics”. I shall describe the following:

1. The inhibitory neurotransmitter GABA is immune suppressive
2. Angiotensin Receptors are in MS Lesions
   ACE inhibition is beneficial in animal models
3. Immune suppressive lipids in the myelin sheath
4. Infamous amyloid proteins provide protection, not harm in neuroinflammatory conditions
5. PPARs are targetable natural “brakes” on neuroinflammation. They may also help explain gender disparity in MS

Rutgers Brain Health Institute

2015-2016

Plenary Seminar Series

No Quiet Surrender: Molecular Guardians In MS Brain

Lawrence Steinman Stanford University

November 12, 2015