Experimental Autoimmune Encephalomyelitis

An invaluable model for multiple sclerosis and type 3 inflammation

T cell signaling and development group

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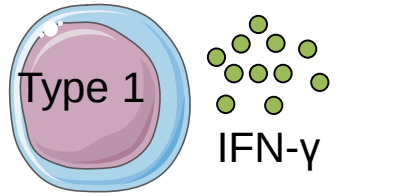
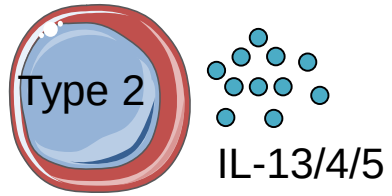
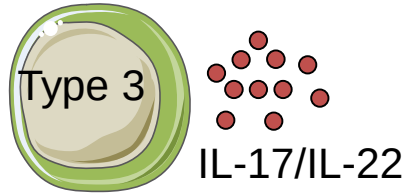
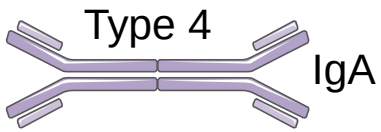
Types of immune response

Type of Immunity	Infections associated with
Type 1	Intracellular viruses/bacteria, tumors
Type 2	Helminths/parasites
Type 3	Extracellular bacteria, fungi
Type 4	Exclusion/prevention

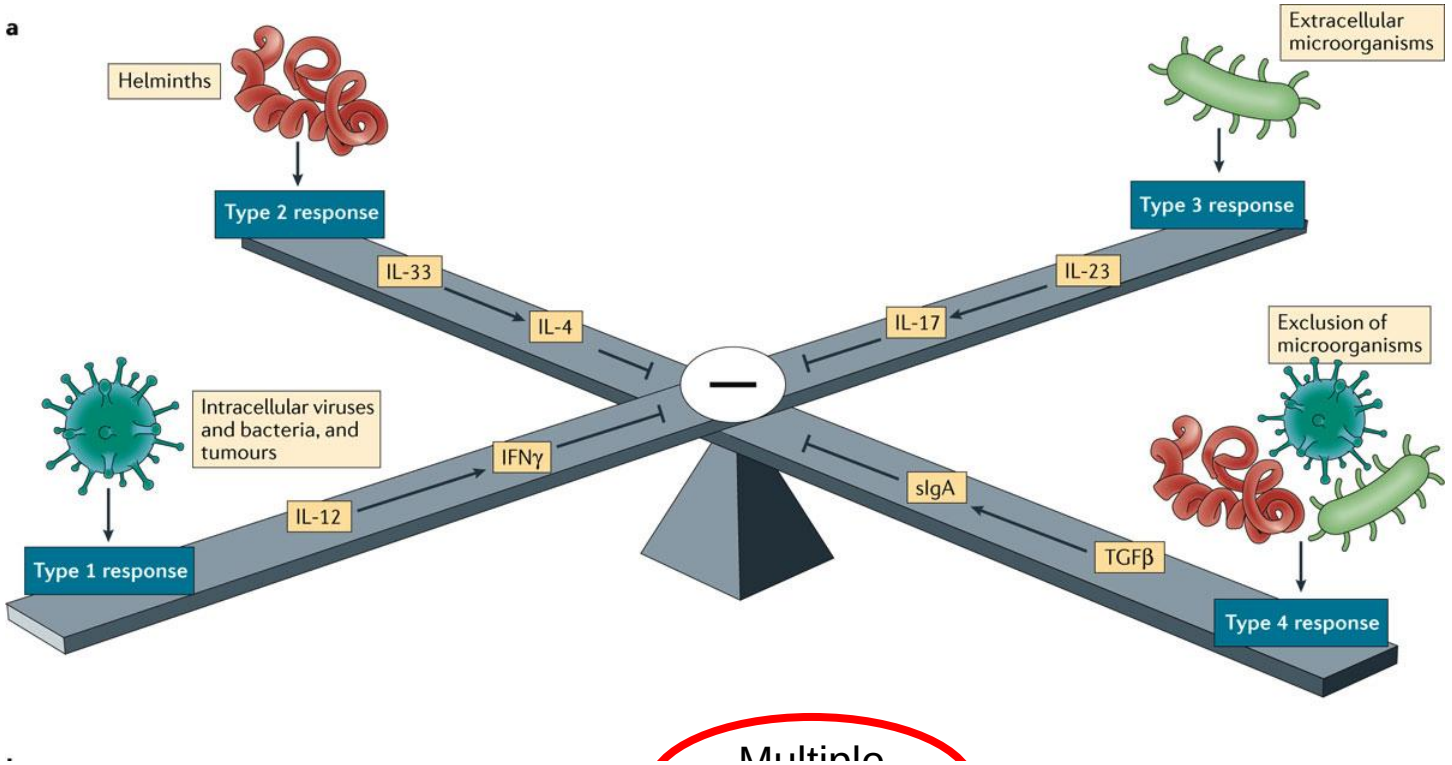








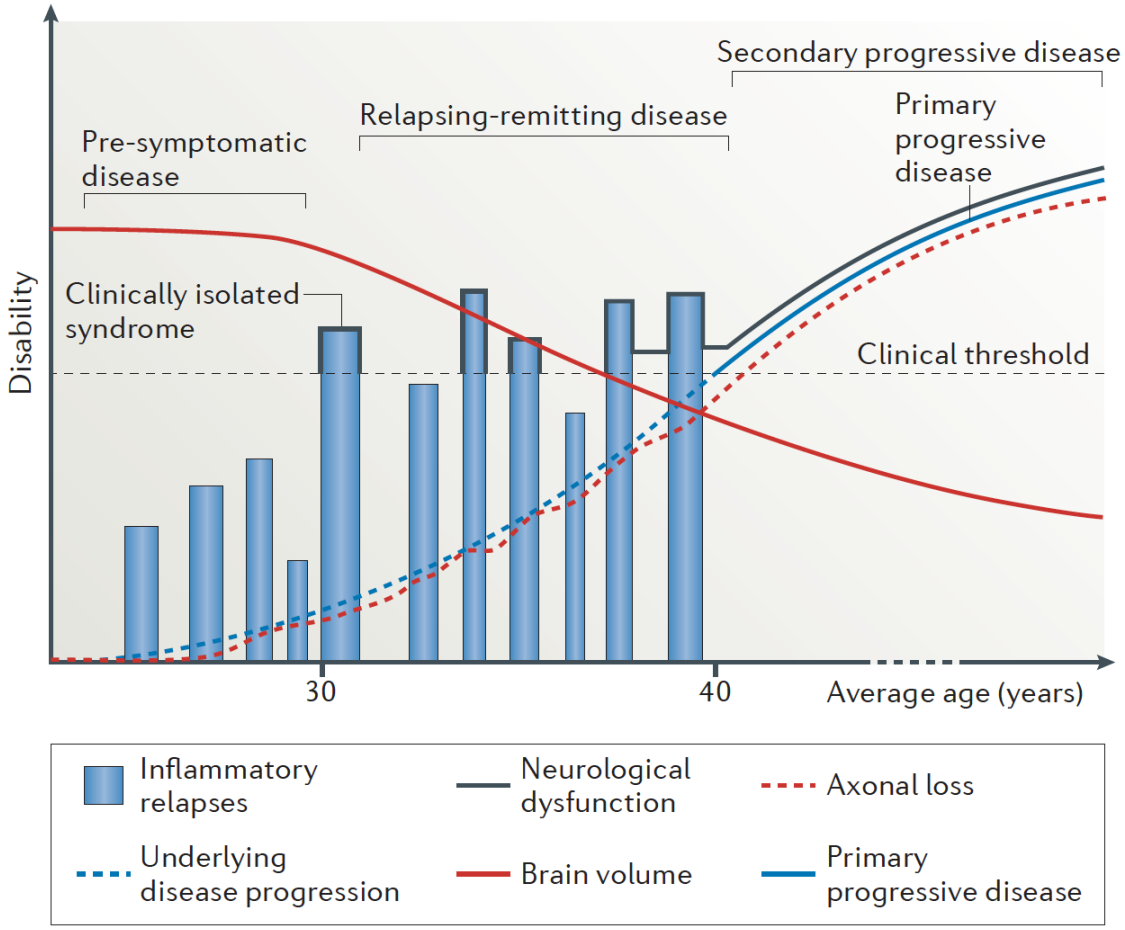
Immunity by equilibrium



Multiple Sclerosis

Approximately 2.5 million people worldwide are afflicted with multiple sclerosis (MS), a chronic neuroinflammatory disease of the brain and spinal cord that is a common cause of serious physical disability in young adults, especially women. MS poses a major personal and socioeconomic burden: the average age of disease onset is 30 years — a time that is decisive for work and family planning — and 25 years after diagnosis, approximately 50% of patients require permanent use of a wheelchair.

Dendrou et al., Nat Rev Imm, 2015

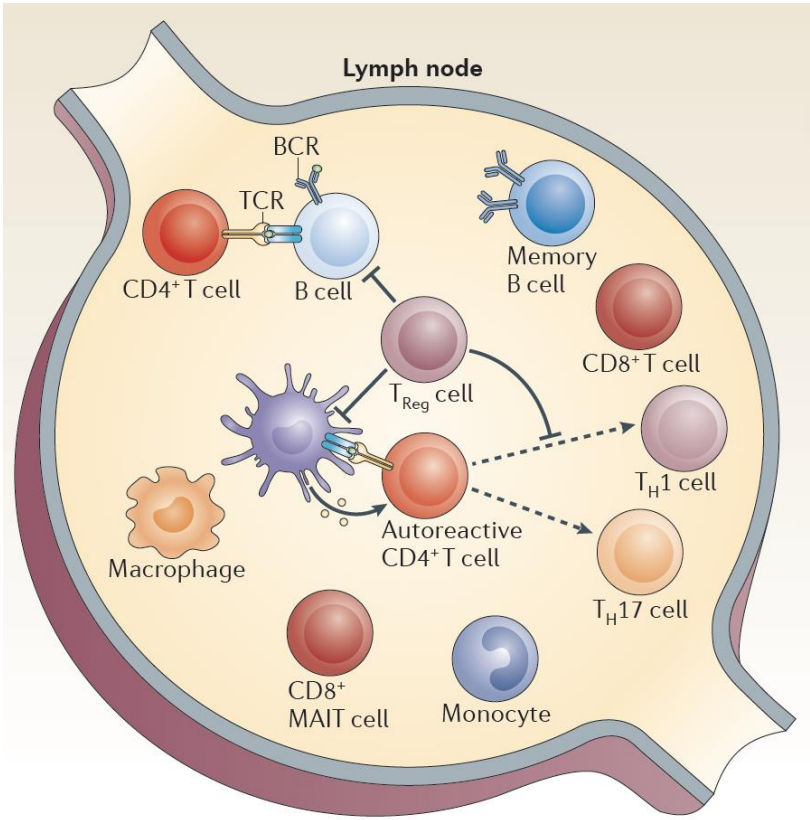


Multiple Sclerosis is an immune mediated disease

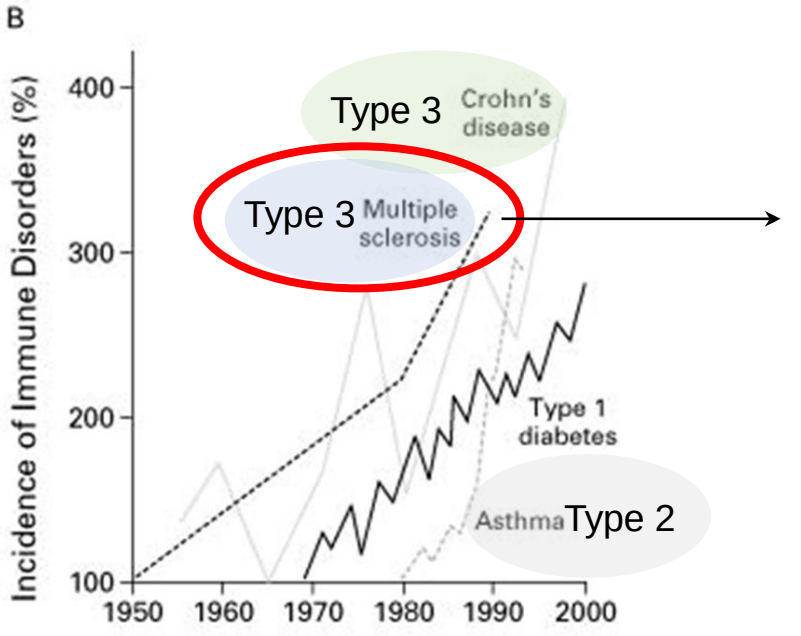
Table 1. Disease-modifying drugs available to patients with RRMS.

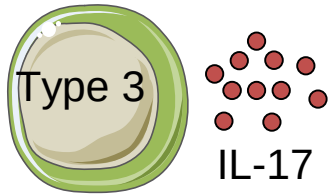
Drug	Brand	Dose	Number of of Injections, Route	Actions
IFN-β1a	Avonex®	7.5 mg 1st dose 15 mg 2nd dose 22.5 mg 3rd dose 30 mg all subsequent doses	1/week, i.m	Balances pro- and anti-inflammatory cytokines Decreases Th17 cells Decreases IL-17
	Rebif®	22 mg or 44 mg	3/week, s.c	
IFN-β1b	Betaseron®	62.5 mg and increase over 6 weeks to 250 mg	1/2 days, s.c	
	Extavia®	62.5 mg and increase over 6 weeks to 250 mg	1/2 days, s.c	
pegIFN-β1a	Plegridy®	63 mg 1st dose 95 mg 2nd dose 125 mg all subsequent doses	1/2 weeks, s.c	
Glatiramer acetate, EKAY	Copaxone®	20 mg or 40 mg	1/day, s.c 3/week, s.c	Blocks pMHC
Dimethyl fumarate	Tecfidera®	240 mg	2–3/day, oral	Anti-inflammatory Anti-oxidative stress
Teriflunomide	Aubagio®	7 or 14 mg	1/day, oral	Inhibits dihydroorotate dehydrogenase, T, B cells and IFN-γ secreting T cells
Fingolimod	Glenya®	0.5 mg	1/day, oral	Antagonist of SIP receptor Decrease T, B cells activates SIP signaling in CNS
Mitoxantrone	Novatrone®	12 mg/m^2	1/3 months up to 2 years	Suppresses T, B cells and macrophages. Reduces Th1 cytokines
Dalfampridine	Ampyra®	10 mg	2/day, oral	Potassium channel blocker Improves motor symptoms, i.e., walking
Humanized Monoclonal Antibody Treatments				
Natalizumab	Tysabr®	300 mg	1/28 days, i.v	Humanized anti-α4-integrin Mab. Affects cell migration, division, growth and survival
Ofatumumab	Arzerra®	3–700 mg	1/2 weeks, i.v	Humanized anti-CD20 Mab. Cytotoxic to CD20+ cells via CDC and ADCC
Ocrelizumab	Ocrevus®	300–600 mg	300 mg weeks 1 and 3, then 600 mg 1/6 months, i.v	Humanized anti-CD20 Mab
Alemtuzumab	Lemtrada®	12 mg	5 days in a row; after 1 year, 3 days	Humanized anti-CD52 Mab. Depletes T, B cells, increases Treg, Th2, decrease Th1 cells
Daclizumab	Zinbryta®	150 mg	1/month, s.c	Humanized anti-CD25 Mab.Blocks IL-2R, decreases T cells, increases NK cells

80% of drugs target immune system

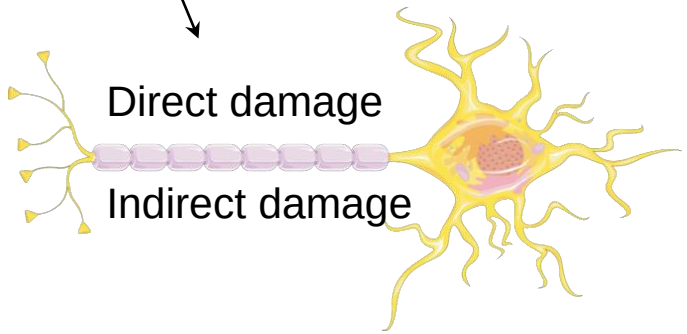


Multiple Sclerosis has a strong type 3 immune signature

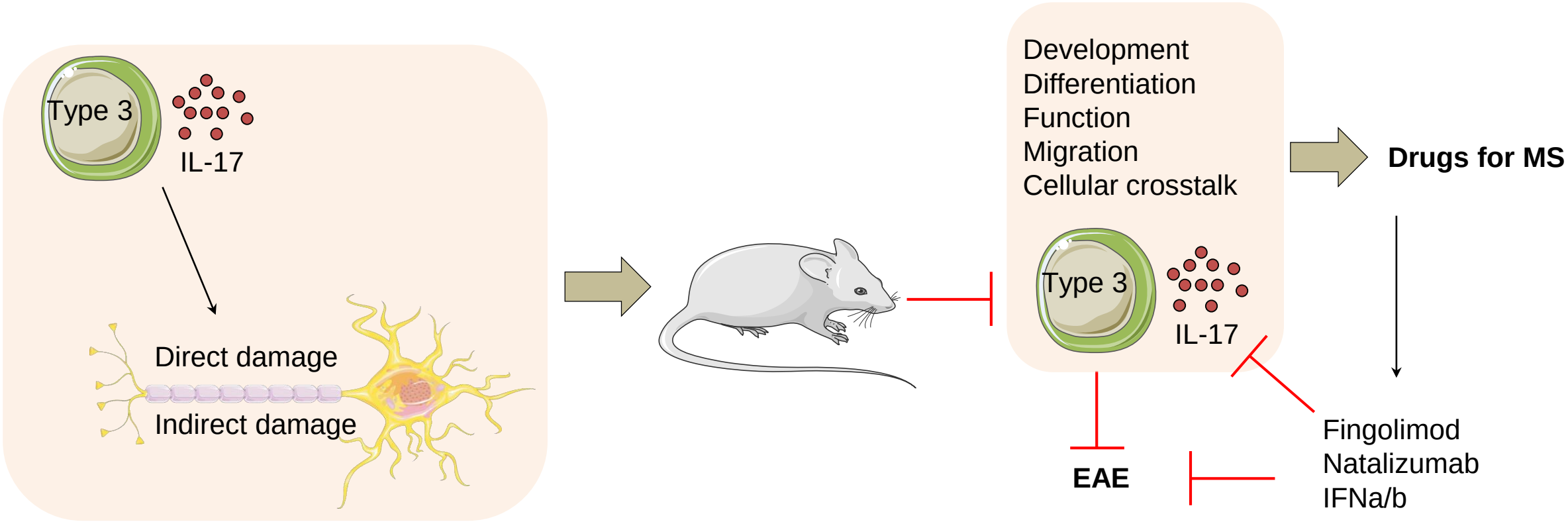




Kaskow & Baecher-Allan, 2018
Schirmer et al, 2013
Hussman et al, 2016
Havrdova et al, 2016
Van Langelaar et al, 2018
Hu et al, 2017



Type 3 immunity of MS is well modelled by EAE



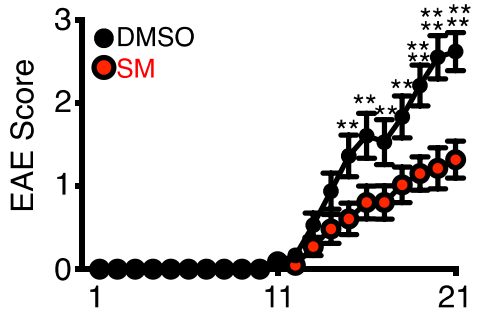
How EAE has helped our research

SCIENCE SIGNALING | RESEARCH ARTICLE

IMMUNOLOGY

SMAC mimetics promote NIK-dependent inhibition of CD4$^+$ T_H17 cell differentiation

John Rizk[1], Joseph Kaplinsky[1], Rasmus Agerholm[1], Darshana Kadekar[1], Fredrik Ivars[2], William W. Agace[1,2], W. Wei-Lynn Wong[3], Matthew J. Szucs[4], Samuel A. Myers[4], Steven A. Carr[4], Ari Waisman[5], Vasileios Bekiaris[1]*



Dialogue in progress

Putative clinical trial

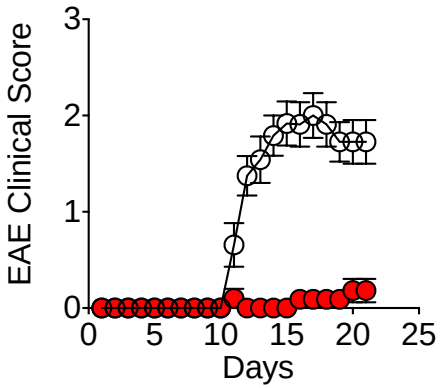
JCI The Journal of Clinical Investigation

The neonatal microenvironment programs innate γδ T cells through the transcription factor STAT5

Darshana Kadekar, … , Richard Moriggl, Vasileios Bekiaris

J Clin Invest. 2020. https://doi.org/10.1172/JCI131241.

Research Article | Immunology

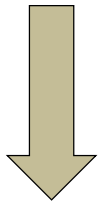


Pre-clinical testing of Novel STAT5 inhibitors

How do we induce EAE?

- There are a few different types of EAE
- Some are genetic and spontaneous
- Others can be passive by transfer of encephalitogenic T cells

- Others require immunization with myelin peptides



MOG-induced EAE

$MOG_{(35-55)}$ = myelin oligodendrocyte glycoprotein

Our MOG-EAE protocol

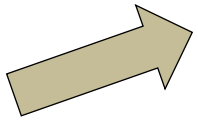
- Group mice and transfer to experimental room to acclimatize
  usually Monday and start Thursday

- At day 0, we mix 50ug of MOG peptide + CFA and make an amalgam
- At day 0, we record each animal's weight
- At day 0, we inject MOG/CFA subcutaneously and pertussis toxin intraperitoneally
- At day 2 we repeat the pertussis toxin injection and put mice in clean cages
- Animals left "untouched" until day 11
  caretakers monitor without handling
- At day 11 we rehouse in clean cages and start weighing and scoring each mouse daily
- At day 21, experiment is terminated for all mice unless a humane endpoint is reached earlier

Scoring system and measures to reduce severity

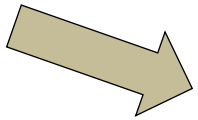
Monitoring happens twice daily:
- (a) morning by caretakers without handling
- (b) late afternoon by scientists; weighing + scoring

- 0.0 = no symptoms
- 1.0 = tail paralysis
- 1.5 = impaired righting reflex or waddled gait
- 2.0 = paralysis of one hind limb
- 2.5 = paralysis of both hind limbs
- 3.0 = weak front limbs with paralyzed hind limbs
- 4.0 = total limb paralysis
- 5.0 = moribund or death



- Provide water in long “nose” bottles
- Provide hydro-gel
- Food pellets in cage
- Moist-absorptive bedding

- Wet food pellets
- Max for 48 hours



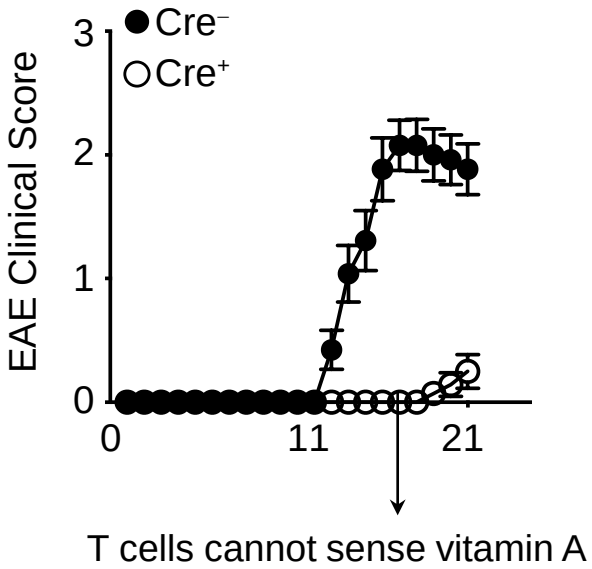
- Humane endpoint

What extra measures are we taking and what more we can do?

- Access to food and water at ground level
  - Often dispersed pellets or hydro-gel packs get buried in bedding
- Hydro-gel is not their favorite
- Need a device to dispense food and water at ground level
- House with healthy animals (which we already do) for thermoregulation
- Heating blankets or extra bedding and nesting material
- High energy treats and foods (raisins, nuts etc)
  - Reluctant due to possible implication in the immune response
- What else?

Increase our confidence into minimal suffering will allow us to benefit more from the EAE model

- Despite its problems EAE is the best we have for MS research
- Ensuring better wellbeing can potentially benefit our science and medical advances



T cells cannot sense vitamin A

- Diabetes and colitis are very common among MS patients
- Infections appear to have a tremendous impact on MS development
- Diet too is can be an important factor in managing MS symptoms

Can we study comorbidities by combining EAE with other models if our confidence in wellbeing increases?

Acknowledgments

Mouse Facilities at DTU

Katrine Fog Starup
- technician and main animal person

Rasmus Agerholm-Nielsen
- post-doc and main EAE person