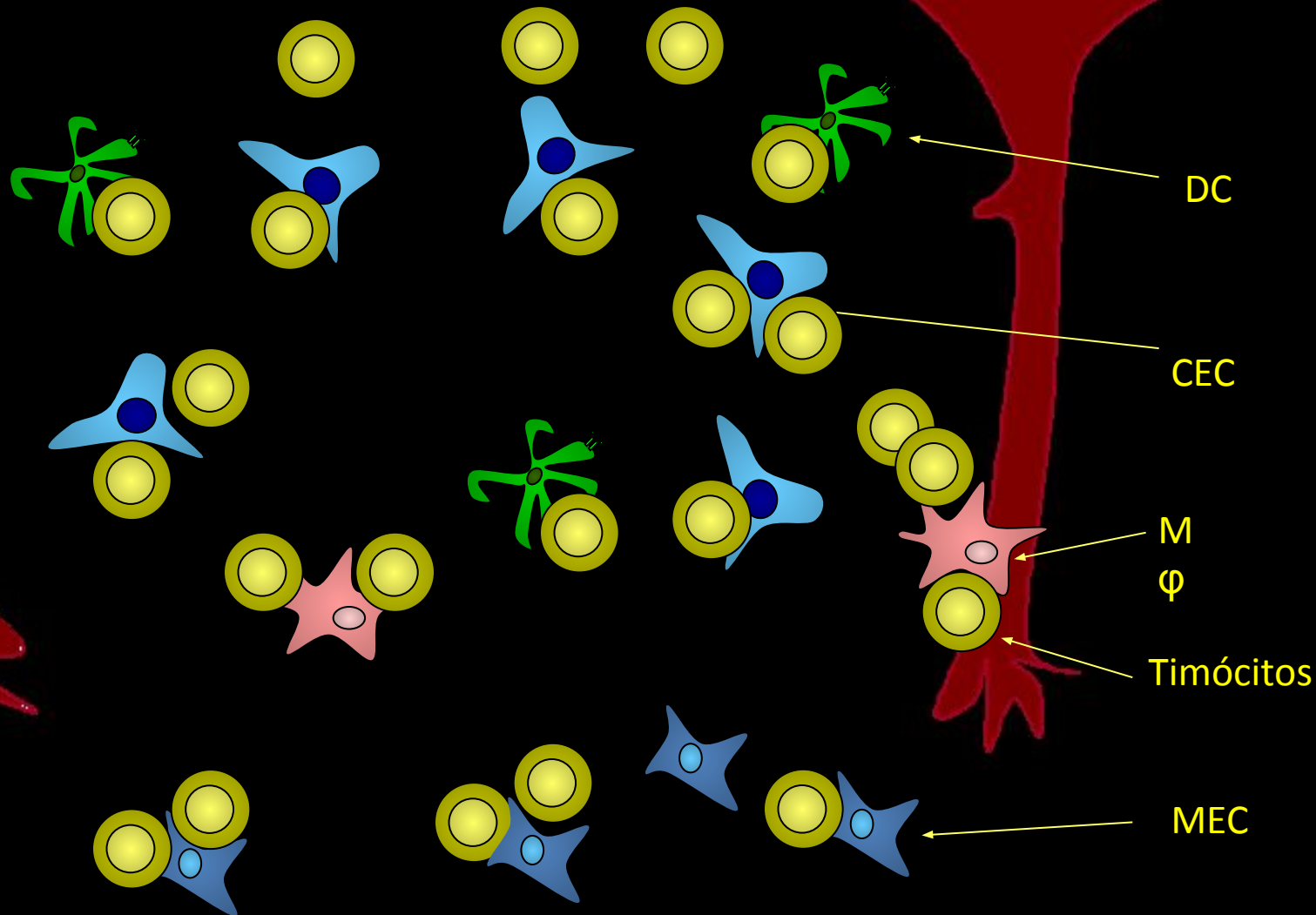


Auto-imunidade Celular

Prof. Dr. Jean Pierre Schatzmann Peron
Laboratório de Interações Neuroimunes

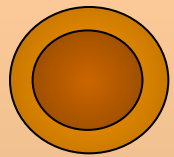
Precursores linfóides, agora chamados timócitos, migram da CÓRTEX PARA MEDULA e não expressam CD4 nem CD8
 $CD4^{neg}$ $CD8^{pos}$

Maturação



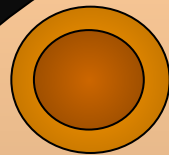
Ao adentrar o timo, timócitos passam a expressar CD4^{pos} CD8^{pos}

Medula



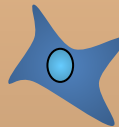
CD4⁻
CD8⁻
TCR⁻

Timo

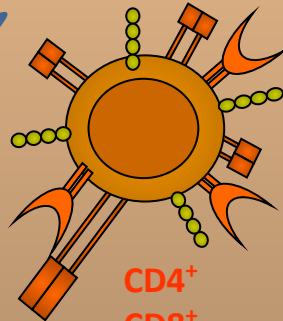


CD4⁻
CD8⁻
Recombinação
TCR
(RAG 1 e 2)

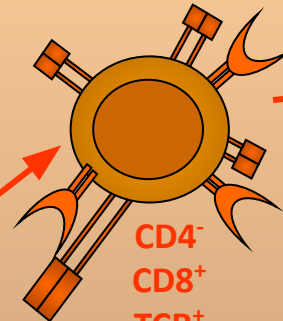
MHCI



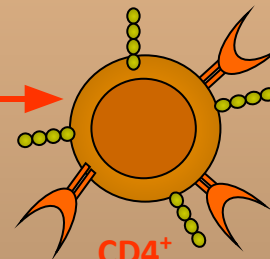
MHCII



CD4⁺
CD8⁺
TCR^{low}

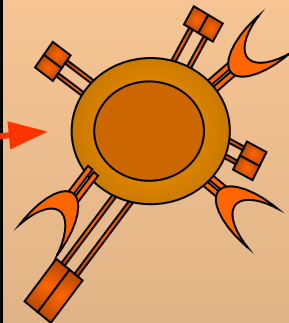


CD4⁻
CD8⁺
TCR⁺

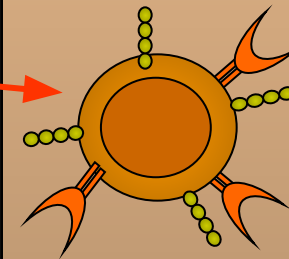


CD4⁺
CD8⁻
TCR⁺

Periferia



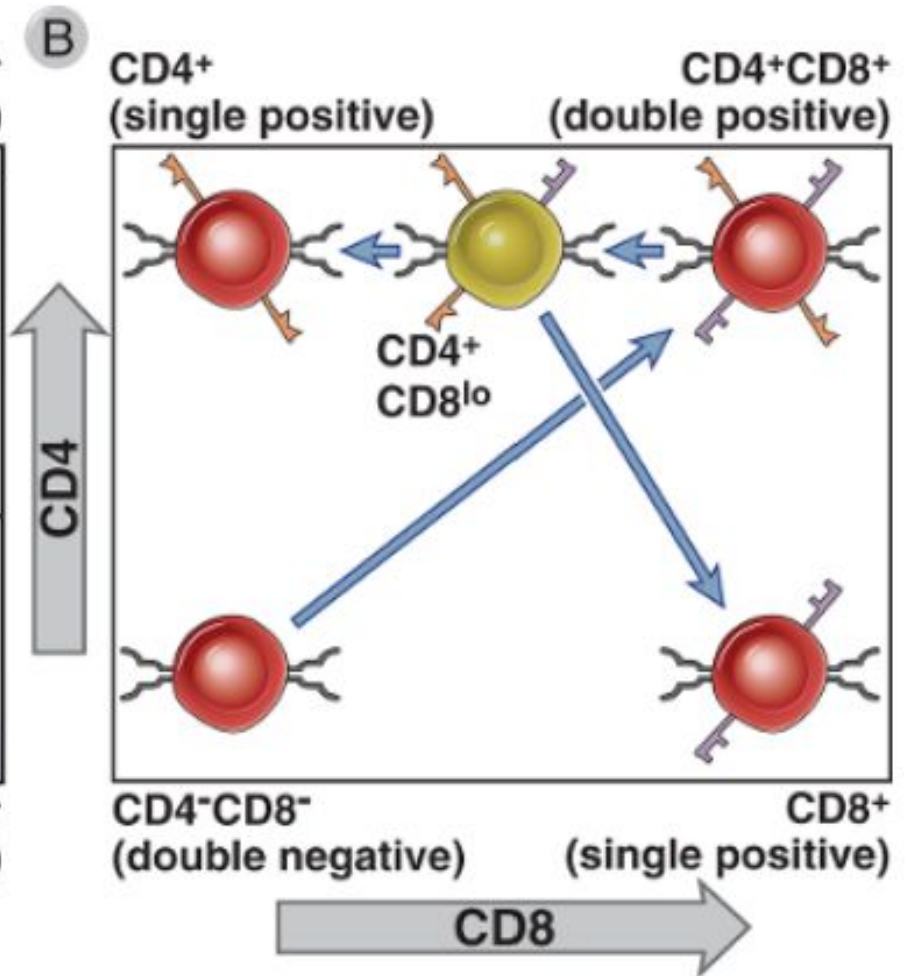
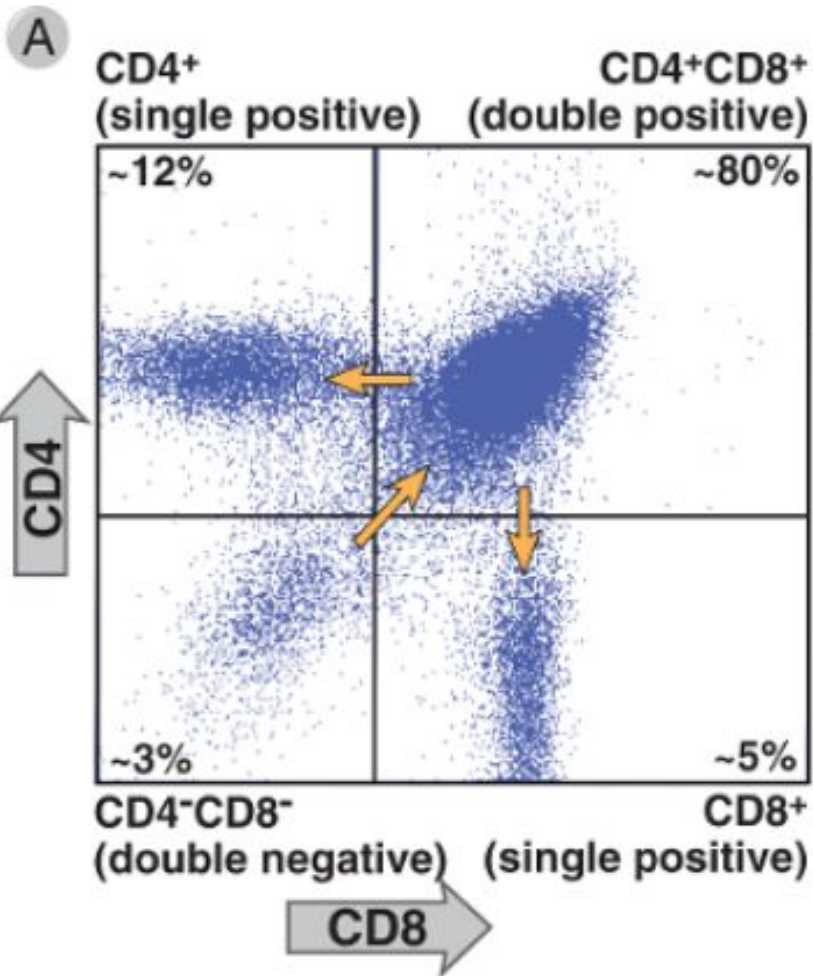
T Citotóxico



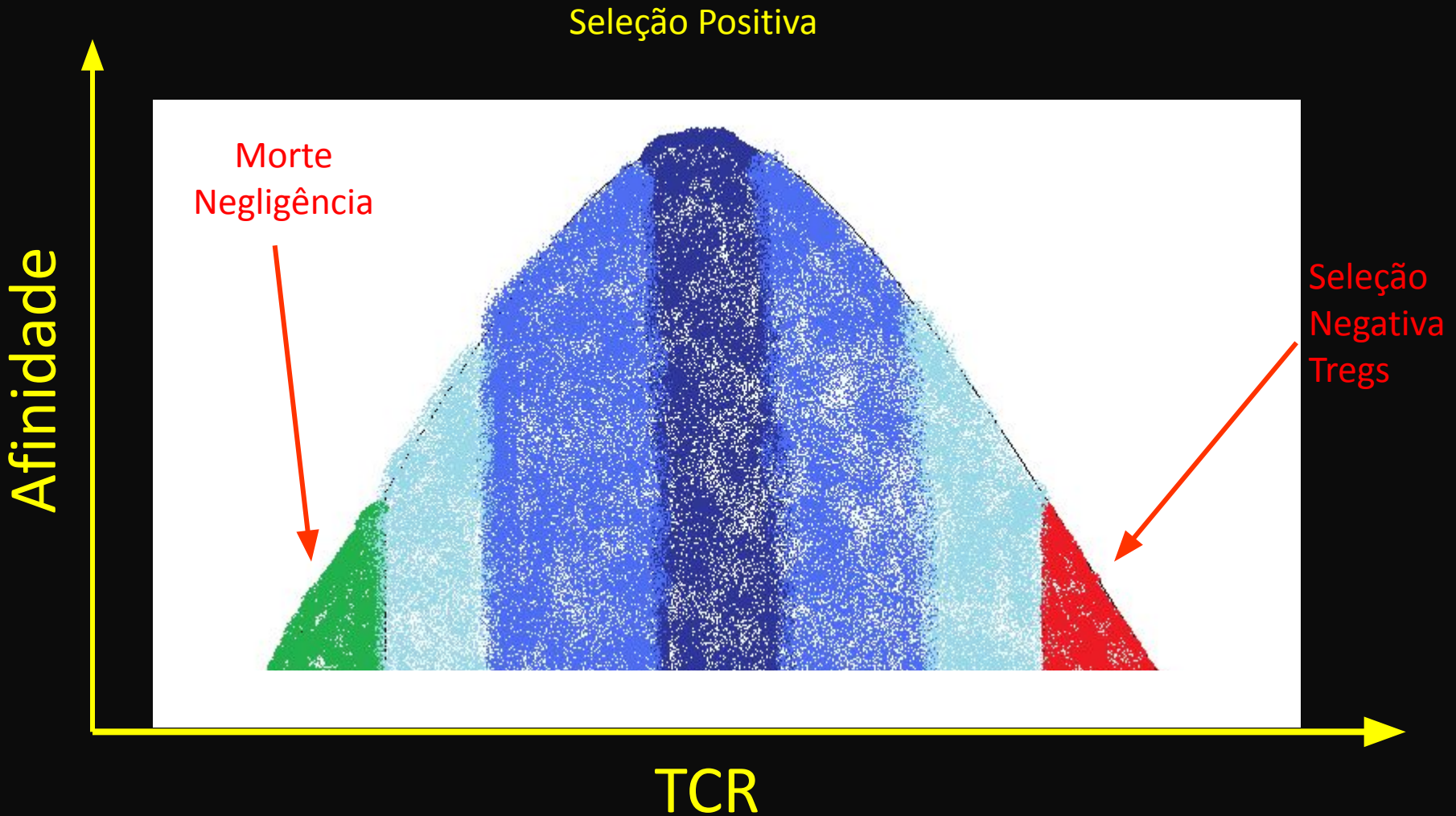
T helper

Córtex

Medula

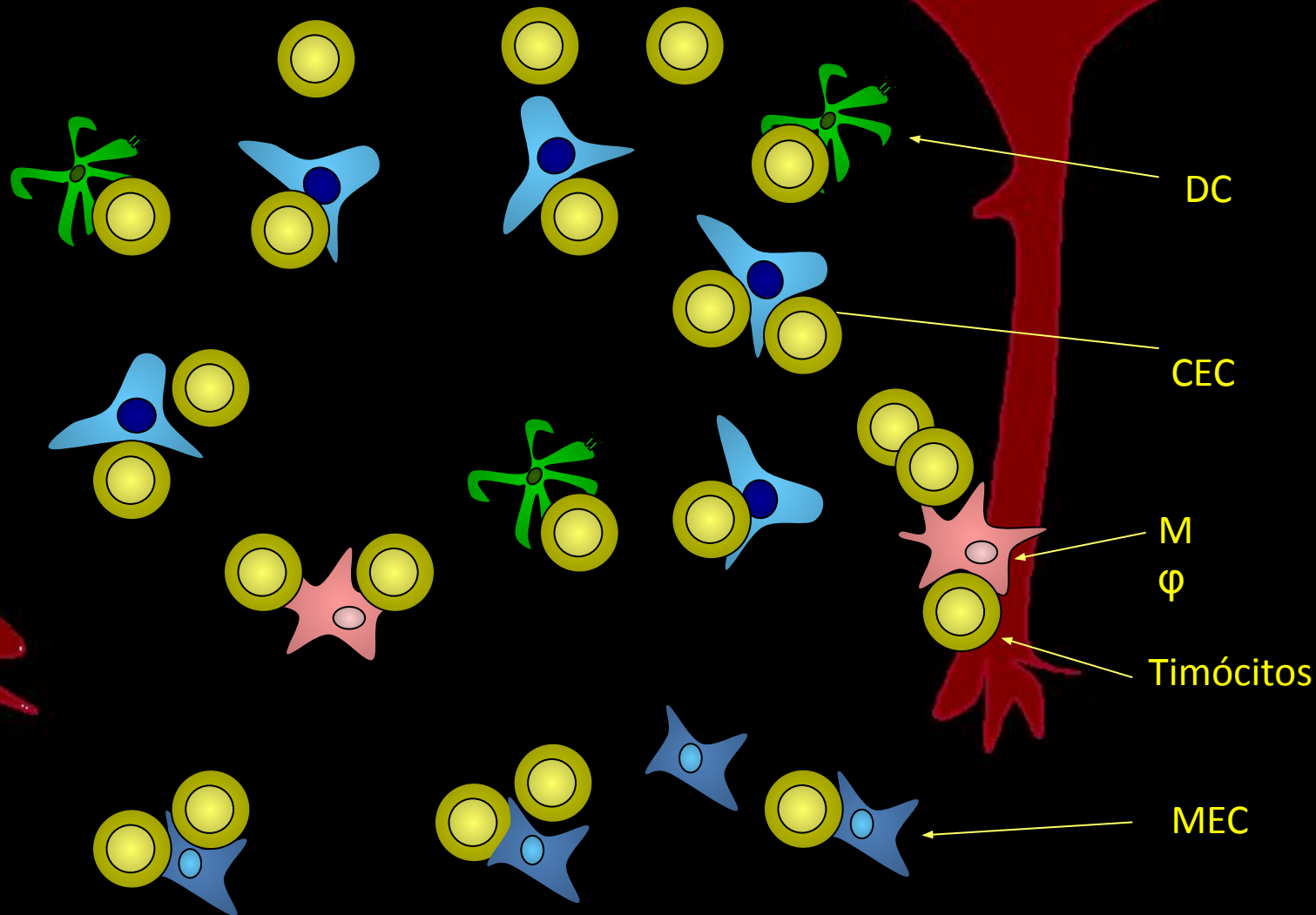


Limiar de Seleção Regido pela Força de Interação Entre os Linfócitos e Antígenos Próprios

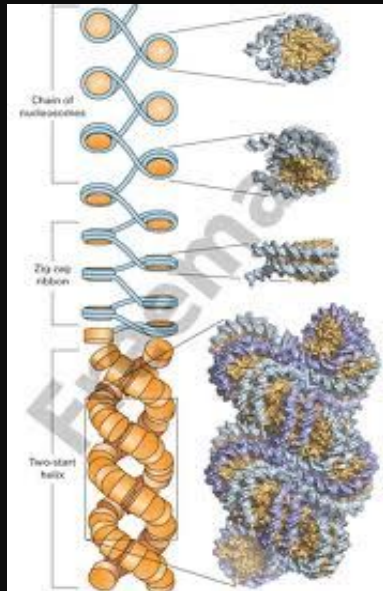


Precursores linfóides, agora chamados timócitos, migram da CÓRTEX PARA MEDULA e não expressam CD4 nem CD8
 $CD4^{neg}$ $CD8^{pos}$

Maturação

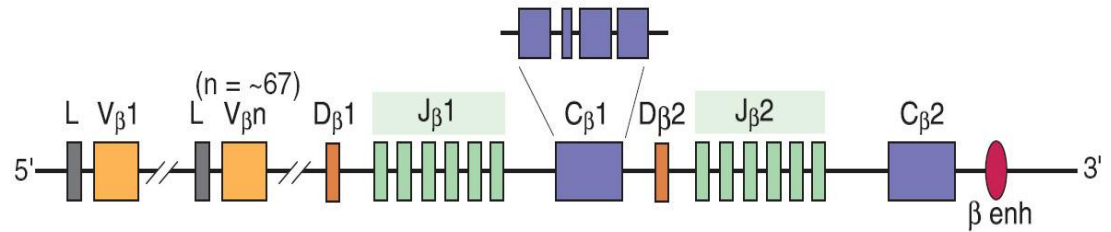


Loci do TCR

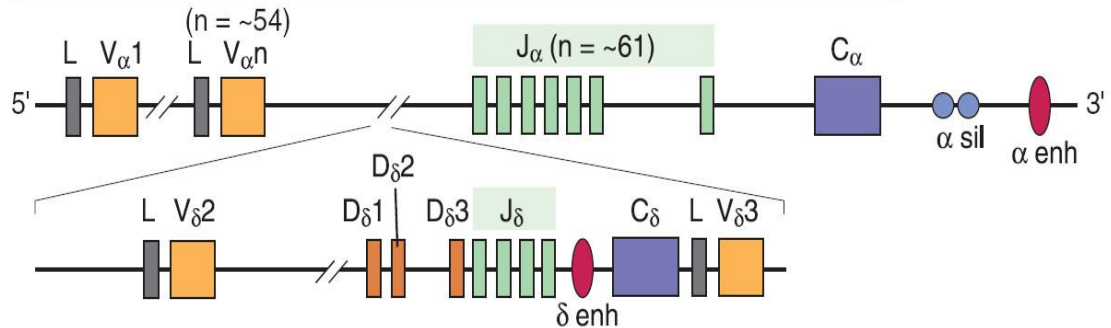


Imunologia Celular e Molecular
ISBN: 9788535222449
Elsevier Editora

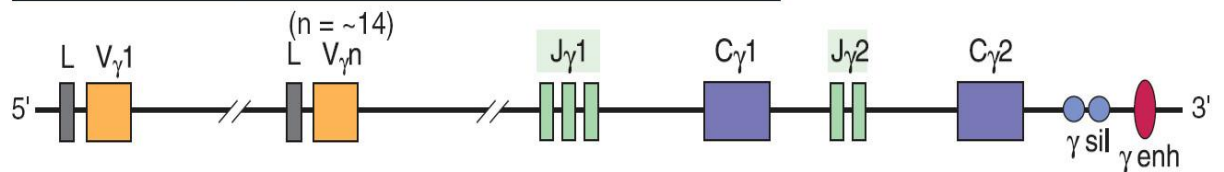
Locus da cadeia β do TCR humano (620 kb; cromossomo 7)

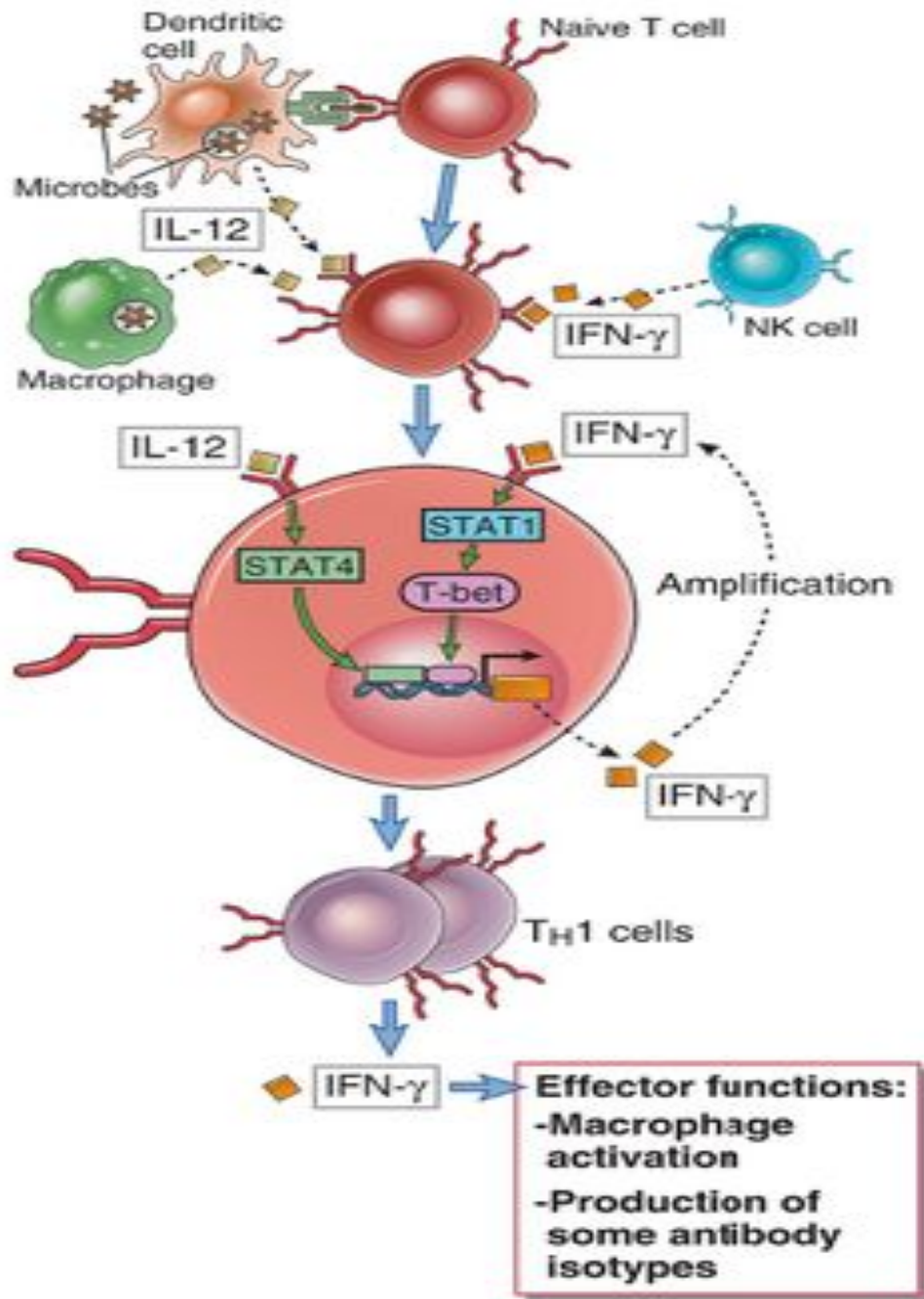


Locus das cadeias α e δ do TCR humano (1.000 kb; cromossomo 14)



Locus da cadeia γ do TCR humano (200 kb; cromossomo 7)

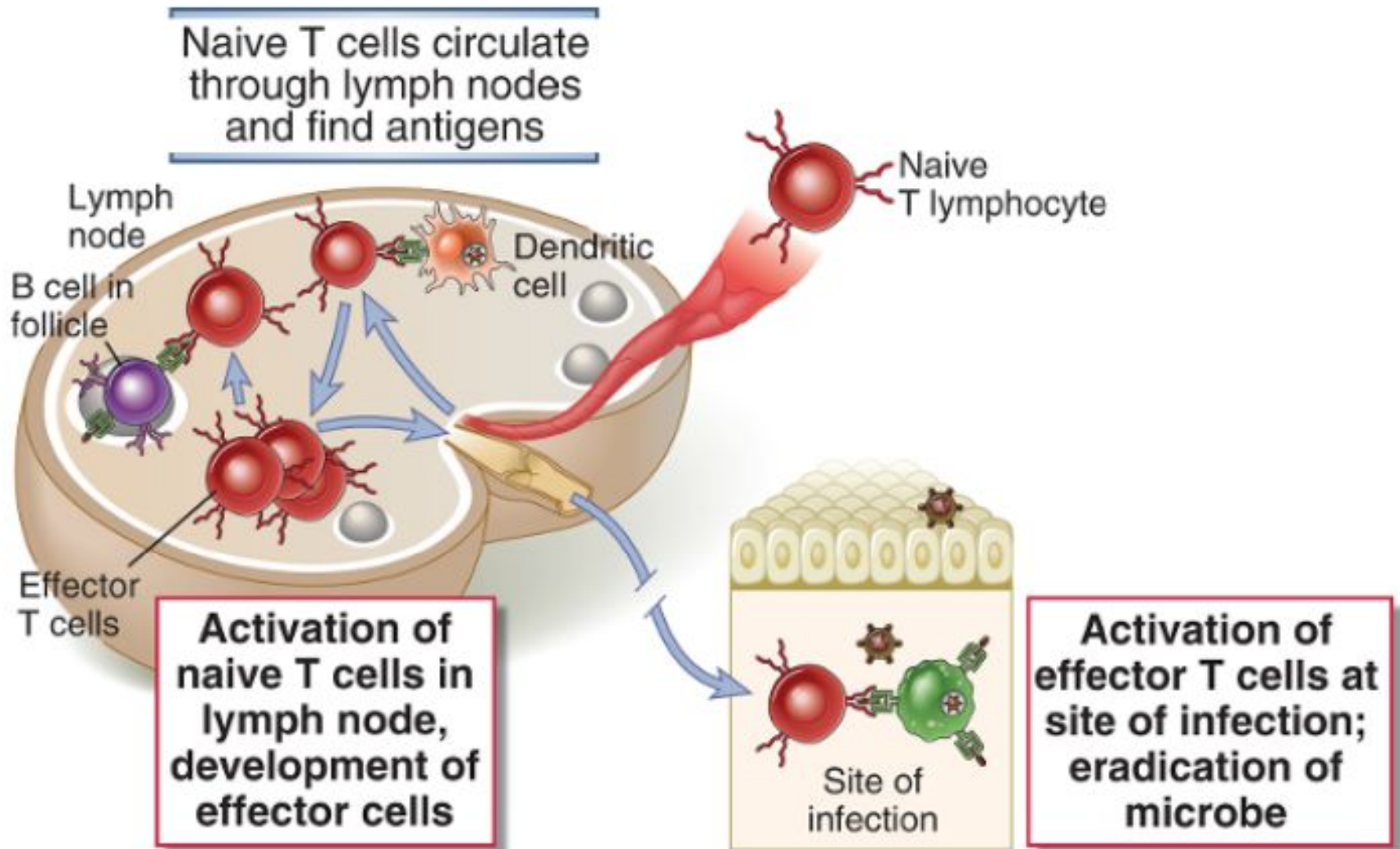




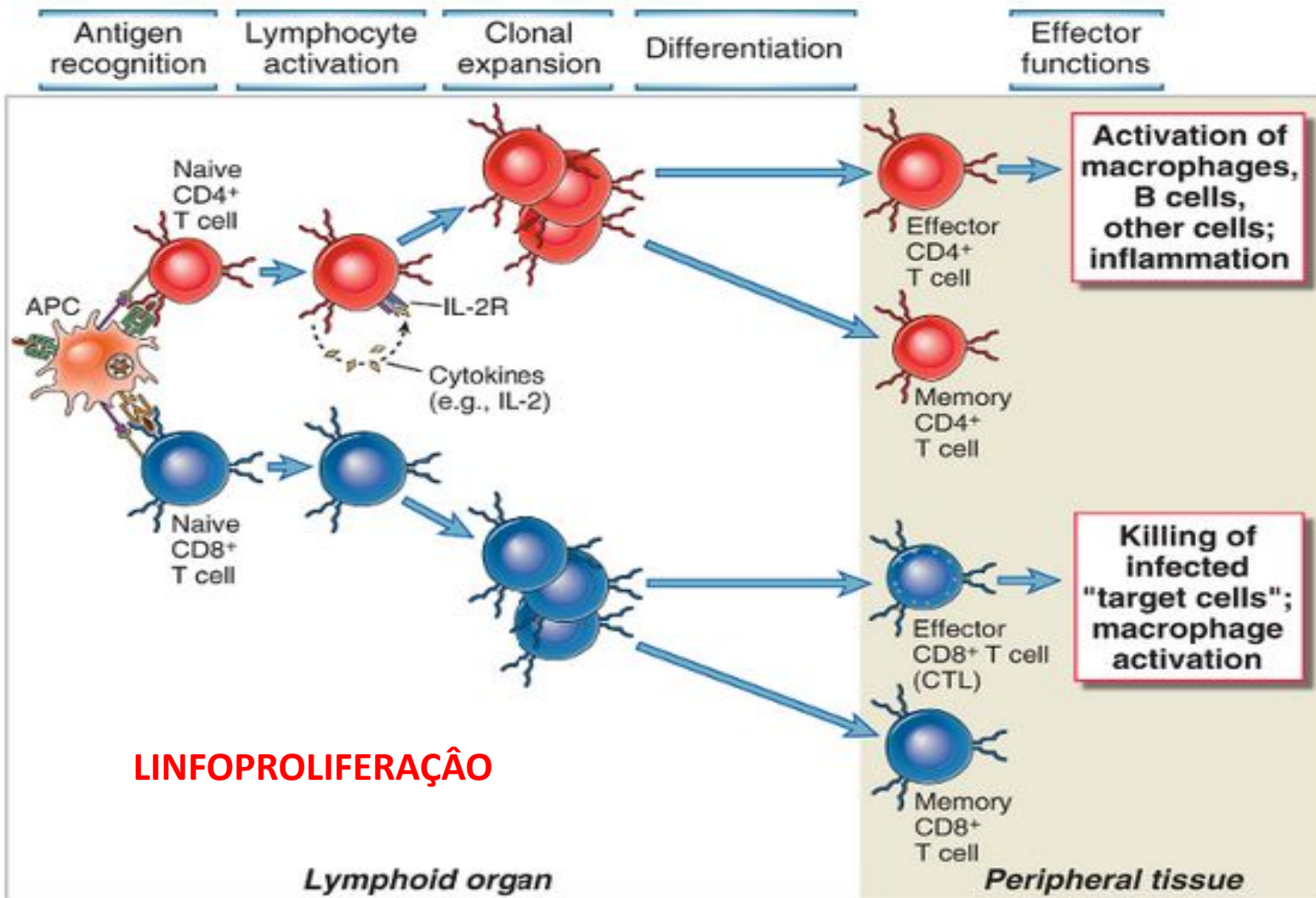
Resposta Th1

- Agentes Intra-celulares
- Ativação da Capacidade Fagocítica e de Degradação Intracelular
- Macrófagos Inflamatórios M1
- A tivação Células NK
- Citocinas principais
- IL-1, IL-12, IL-8, IL-18
- IL-12, TNF- α , IFN- γ

Apresentação de Antígenos e Ativação dos Linfócitos T



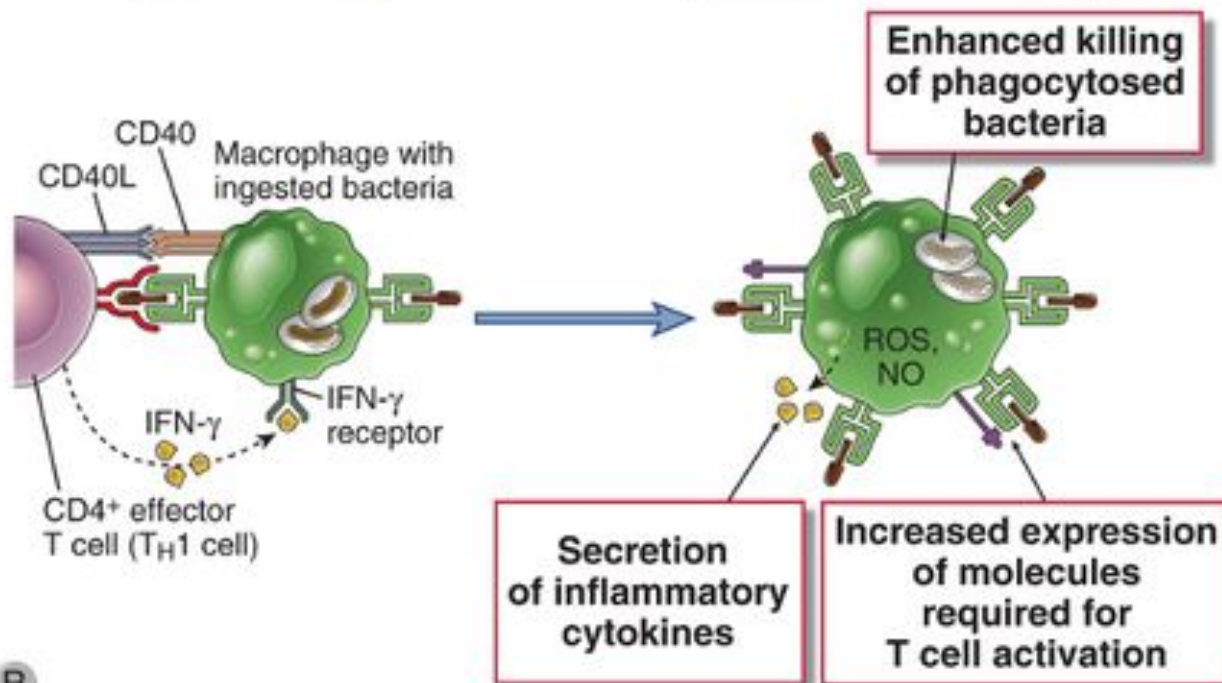
Quais eventos celulares são observados?



A

Activation of macrophages

Responses of activated macrophages

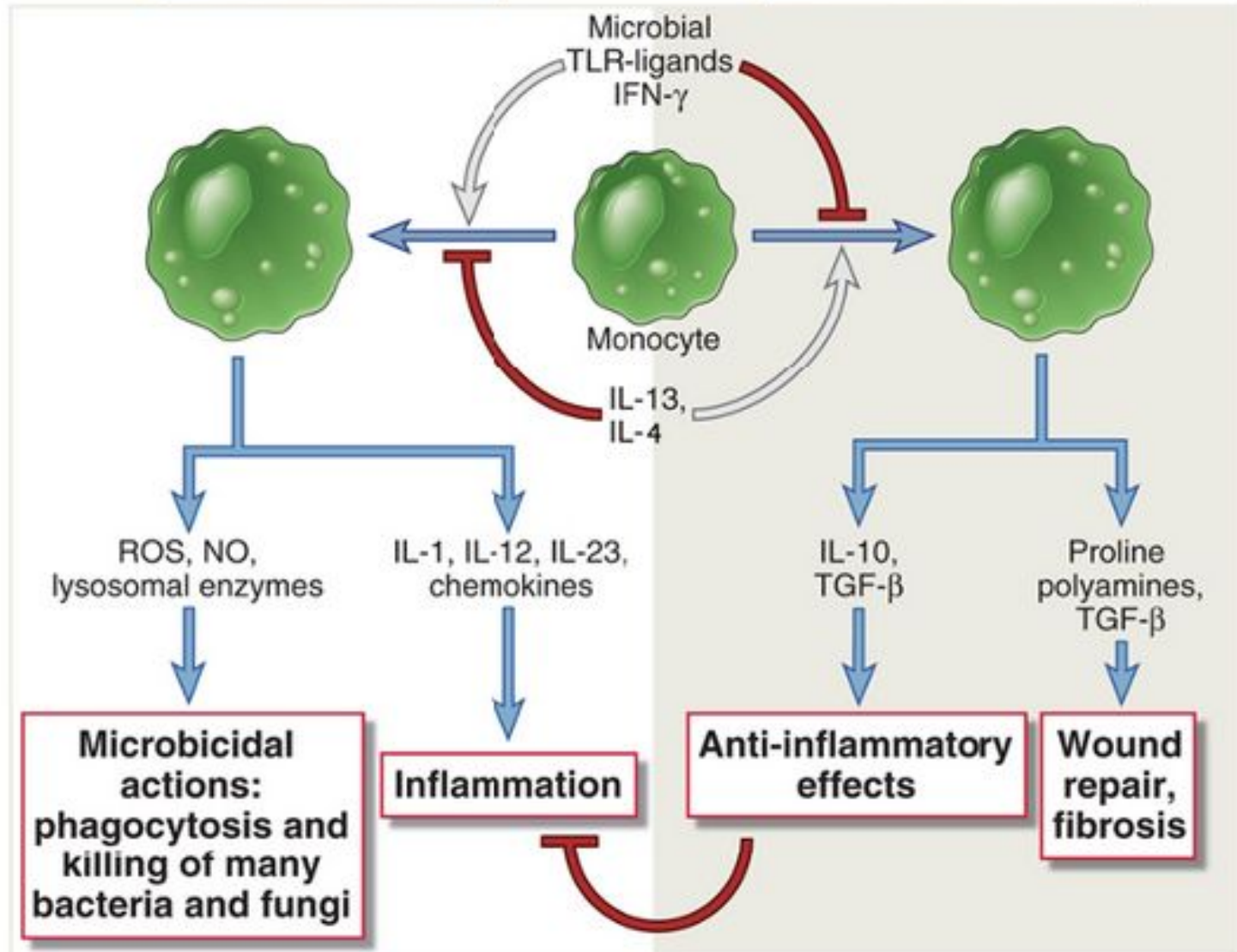


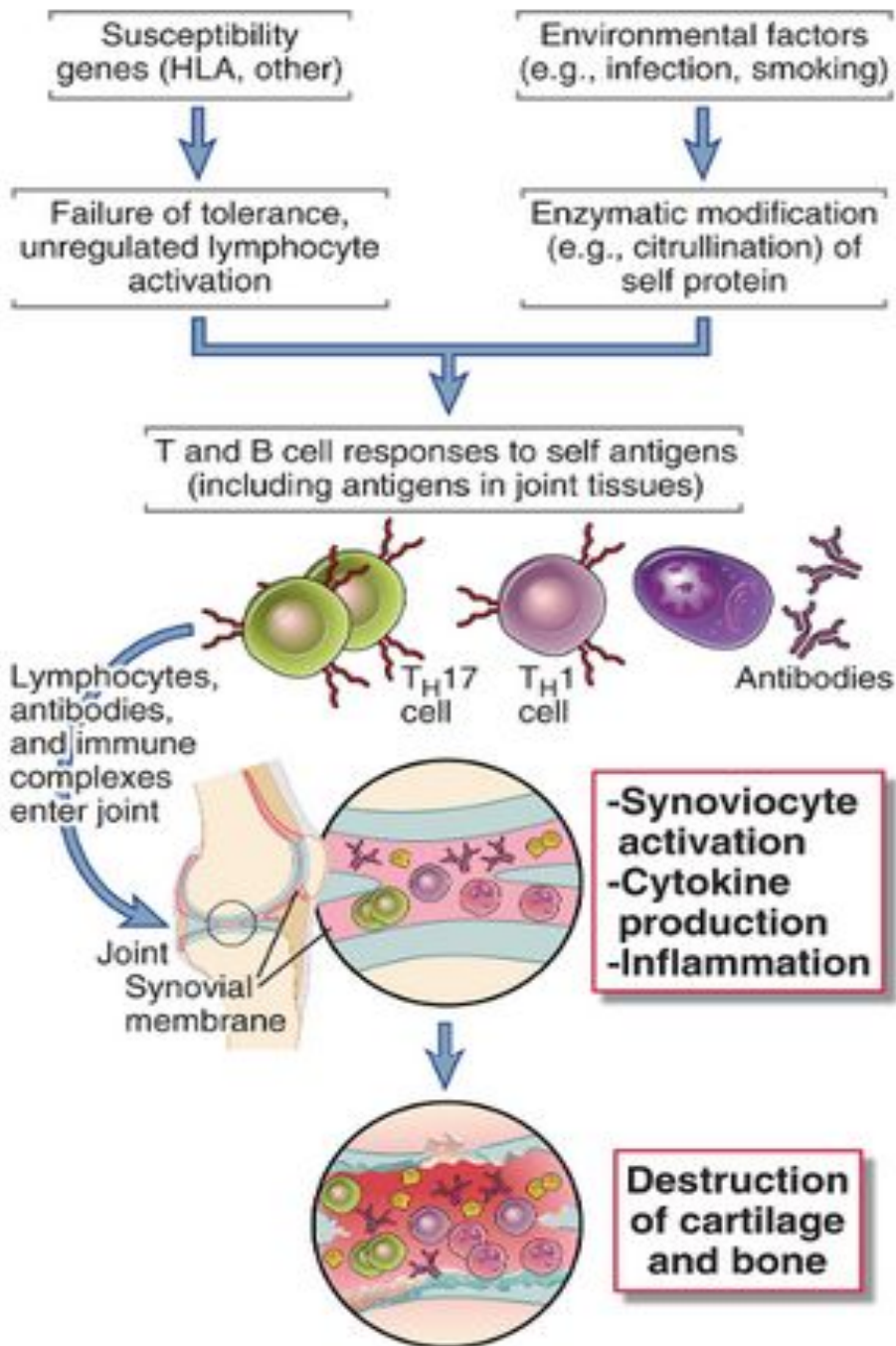
B

Macrophage response	Role in cell-mediated immunity
Production of reactive oxygen species, nitric oxide, increased lysosomal enzymes	Killing of microbes in phagolysosomes (effector function of macrophages)
Secretion of cytokines (TNF, IL-1, IL-12) and chemokines	TNF, IL-1, chemokines: leukocyte recruitment (inflammation) IL-12: T _H 1 differentiation, IFN-γ production
Increased expression of B7 costimulators, MHC molecules	Increased T cell activation (amplification of T cell response)

Classically activated macrophage (M1)

Alternatively activated macrophage (M2)





Tipo IV

Antígenos Protéicos

Apresentados

aos Linfócitos T

Th1

Th17

TABLE 18–4 T Cell–Mediated Diseases

Disease	Specificity of Pathogenic T Cells	Principal Mechanisms of Tissue Injury
Rheumatoid arthritis	Collagen? Citrullinated self proteins?	Inflammation mediated by T _H 17 (and T _H 1?) cytokines Role of antibodies and immune complexes?
Multiple sclerosis	Protein antigens in myelin (e.g., myelin basic protein)	Inflammation mediated by T _H 1 and T _H 17 cytokines Myelin destruction by activated macrophages
Type 1 diabetes mellitus	Antigens of pancreatic islet β cells (insulin, glutamic acid decarboxylase, others)	T cell–mediated inflammation Destruction of islet cells by CTLs
Inflammatory bowel disease	Enteric bacteria Self antigens?	Inflammation mediated by T _H 17 and T _H 1 cytokines
Autoimmune myocarditis	Myosin heavy chain protein	CTL-mediated killing of myocardial cells Inflammation mediated by T _H 1 cytokines

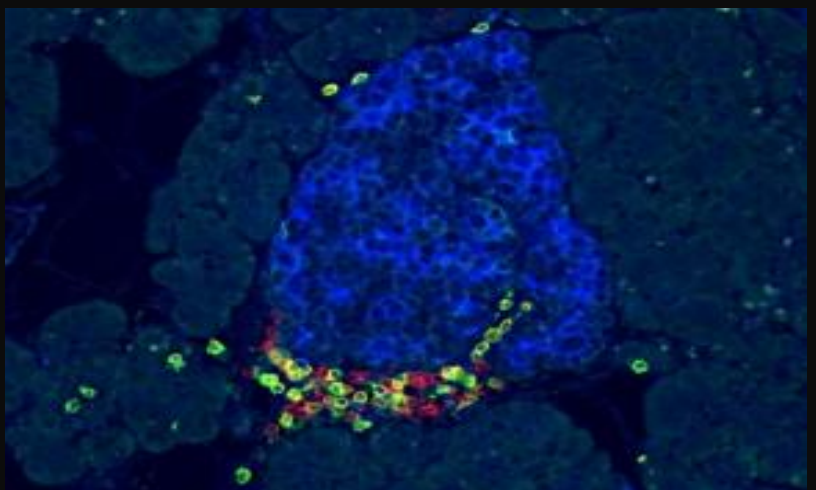
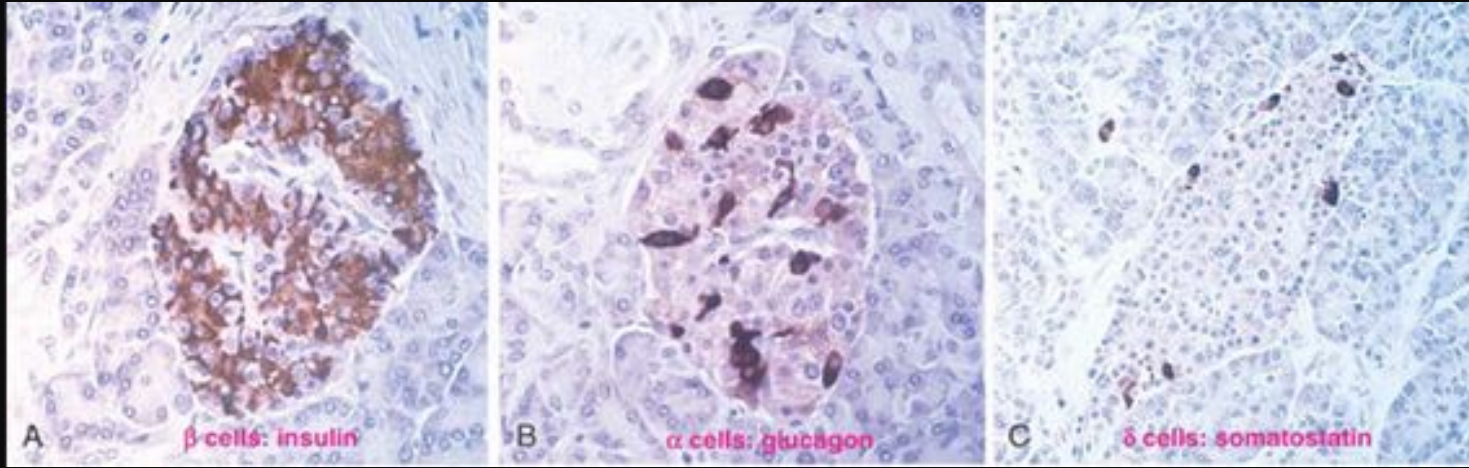
Examples of human T cell–mediated diseases are listed. In many cases, the specificity of the T cells and the mechanisms of tissue injury are inferred on the basis of the similarity with experimental animal models of the diseases.

Diabetes Tipo I



- Willy Gepts -
- USA – 30.000 novos casos por ano
- HLA – DR3 e HLA-DR4
- Não HLA- Polimorfismo no gene insulina
- Auto-antígenos
 - Anti-insulina
 - Islet cell antigen 512
 - GAD (descarboxilase do ácido glutâmico)
 - Mimetismo molecular – Coxsackie vírus B, reovírus 1b, rubéola
- Experimentalmente a transferência de linfócitos T CD4 ou T CD8 induzem a doença
- Linfócitos B ou anticorpos não!

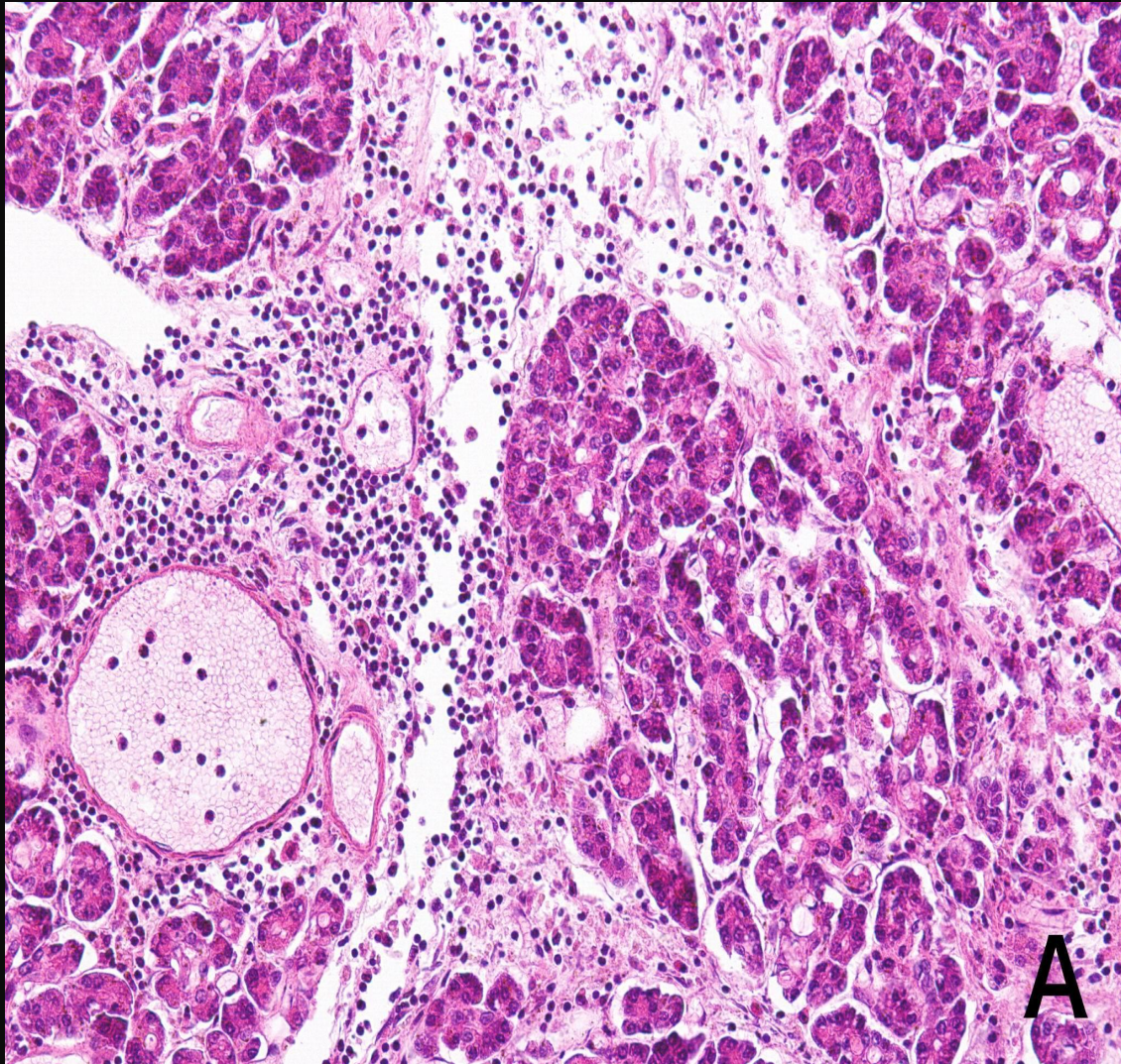
Imunopatologia – Na fase inicial é observado infiltrado inflamatório de linfócitos – macrófagos - INSULITE



CD3 CD8

Table 1. Classification and observations on types of diabetes mellitus

Type	Characteristic	Clinical comment
Type 1	Autoimmune, previously called juvenile or insulin-dependent diabetes mellitus	Potential association with other autoimmune diseases
Type 2	Polygenic and influenced by environment	Increasing incidence associated with higher life span and western cultural habits
Gestational	Aggressive clinical progress	May persist after pregnancy
Secondary	Side effect of medications or pancreas dysfunction (e.g.; steroids, chronic alcoholism)	Causative disease or medication may also influence ocular and lacrimal function
Genetic	Genetic defects in insulin secretion or action	Potential ocular associated malformations

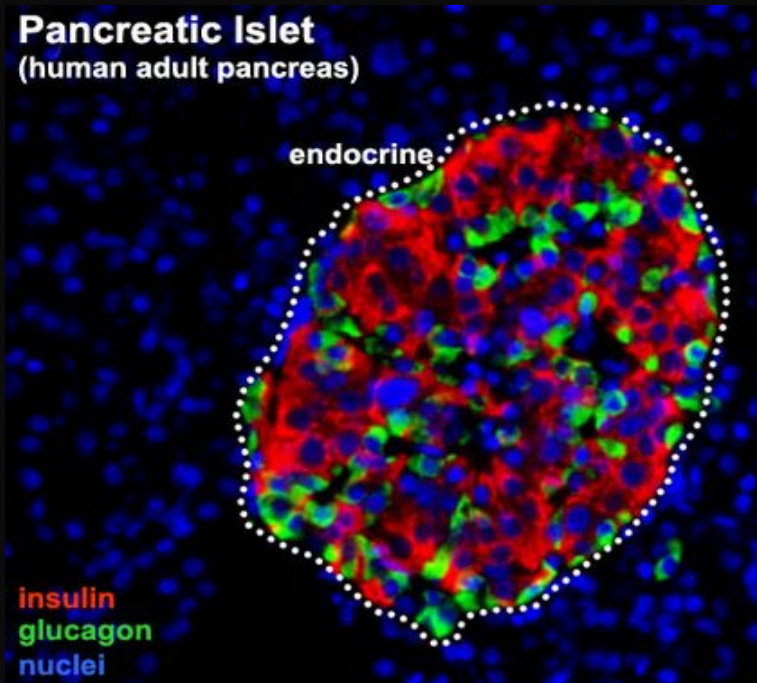


Diabete
fulminante

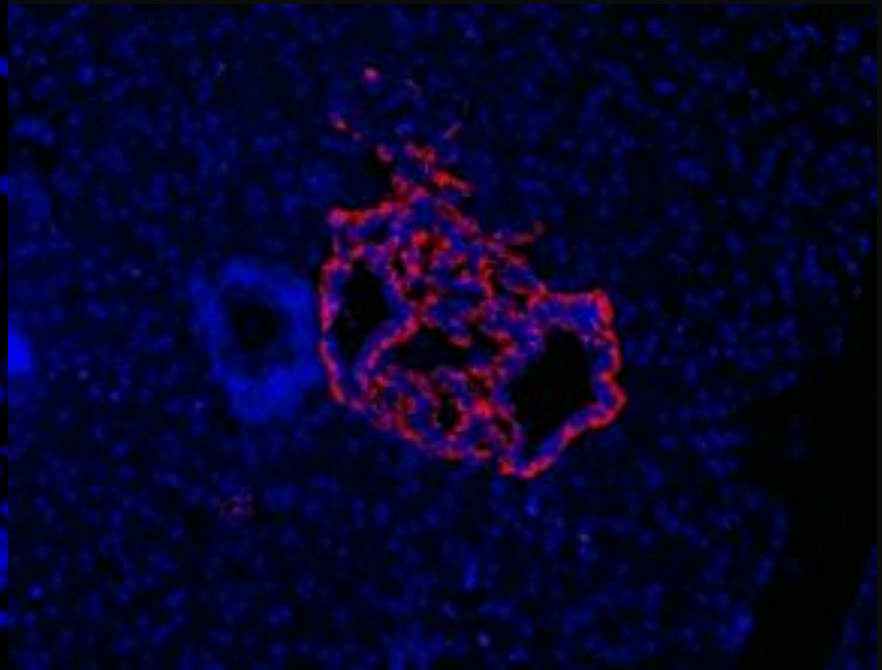
infiltrado
inflamatório e
fibrose

Imunopatologia – Na fase crônica da doença, devido à ampla destruição das ilhotas e redução do Ag, há uma grande regressão do infiltrado inflamatório

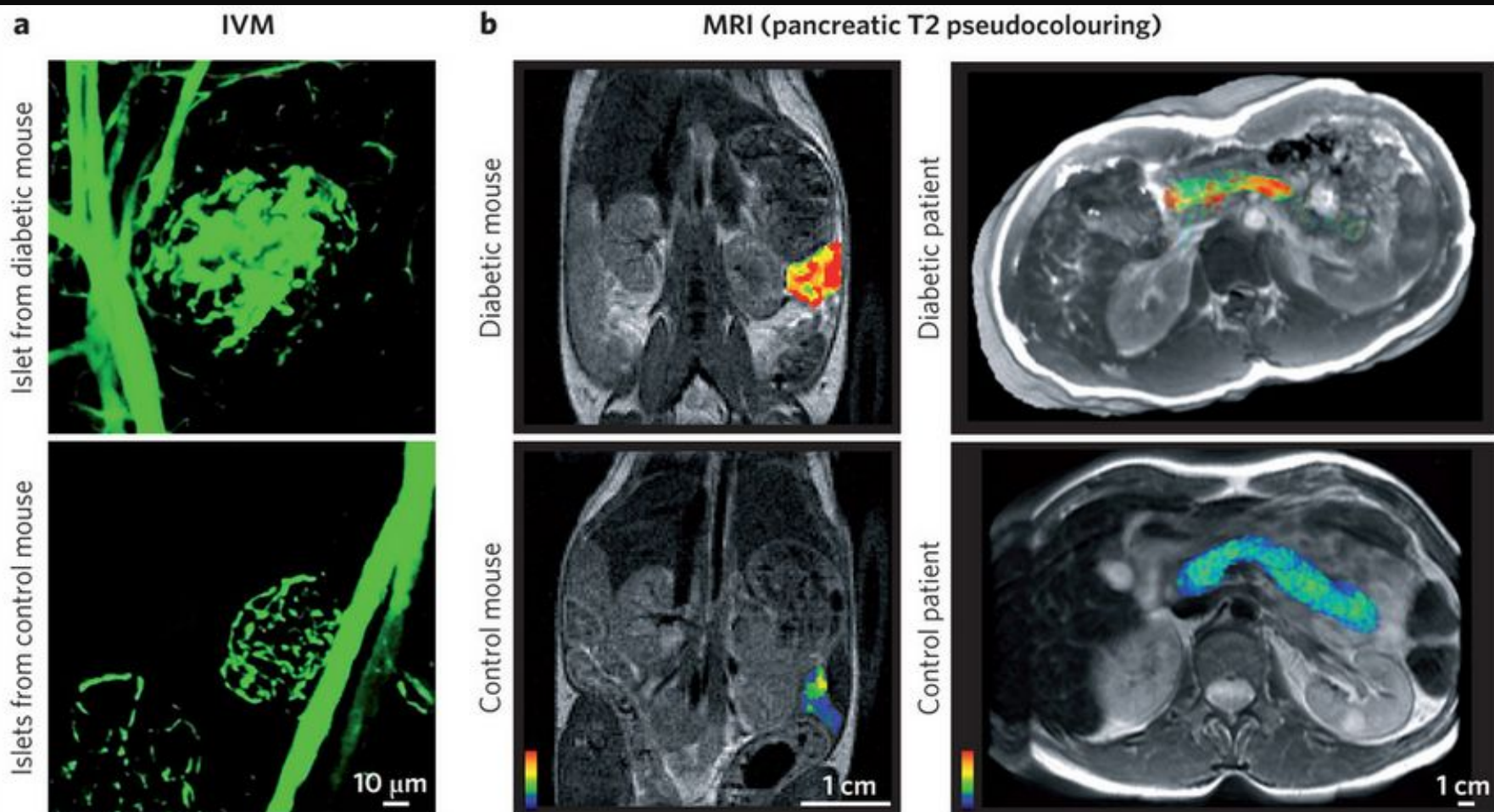
Ilhotas Controle



Paciente Diabético
19 anos evolução



While >70% of young patients with acute onset of the disease show insulinitis (in 5-10% of their islets), the lesion is present in only 4% of patients one year after the diagnosis is made. The inflammation disappears together with the remaining beta cells, leaving small islets that are devoid of any insulin-positivity – Redução progressiva do antígeno insulina – Ilhotas residuais podem permanecer.



a, During type 1 diabetes development, islets of Langerhans contain inflammatory cells (insulinitis) and show increased microvasculature and permeability, shown here by intravital imaging (IVM) during the perfusion phase of a dextran nanoparticle (crosslinked iron oxide nanoparticles labelled with fluorescein isothiocyanate)¹⁵⁸. At later phases, nanoparticles accumulate in macrophages in insulinitis islets. **b**, Changes in T2, indicative of nanoparticle accumulation in insulinitis, are pseudocoloured and superimposed onto T1 images. In both mice¹⁵⁹ and human patients¹⁶⁰, there is a clear difference between type 1 diabetes (top) and normal individuals (bottom).

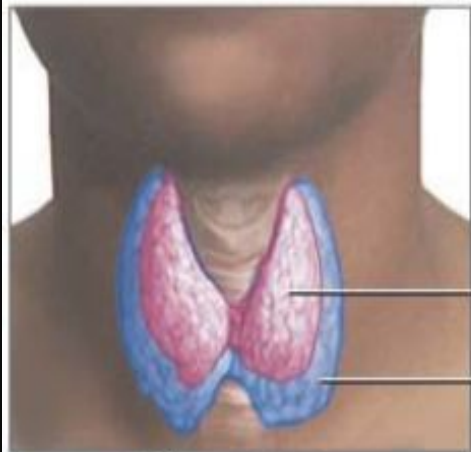
Tireoidites – Doença de Graves

- HLA – DR3
- Auto-antígenos
 - Receptor TSH – porção extracelular, porém anticorpos contra as porções transmembrana já foram detectados
 - Anti-TPO (Tireoperoxidase) – não costumam ser patogênicos
- Mimetismo molecular – Vírus, *Yersinia enterocolítica*
- Resposta Th1-Th17 – citocinas pró-inflamatórias IL-1, IL-4, IL-5, IL-8, IL-12, IFN- γ , TNF- α ...

Doença de Graves



Exophthalmos (bulging eyes)



Diffuse goiter

Normal

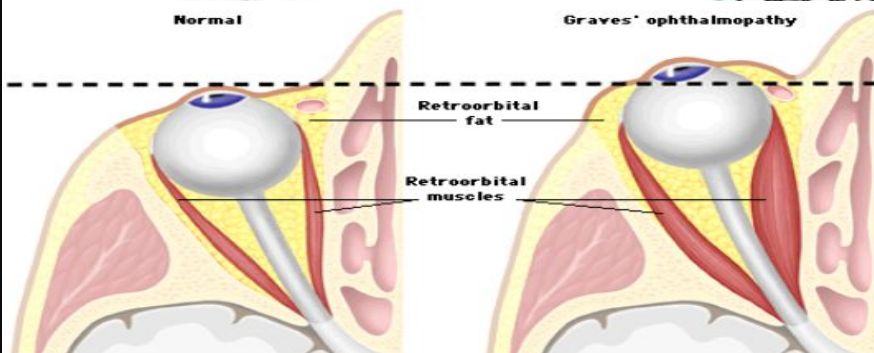
Graves' disease is a common cause of hyperthyroidism, an over-production of thyroid hormone, which causes enlargement of the thyroid and other symptoms such as exophthalmos, heat intolerance and anxiety

Normal thyroid

Enlarged thyroid

ADAM

Graves' ophthalmopathy



Retroorbital fat

Retroorbital muscles



A. Normal



B. Graves' disease



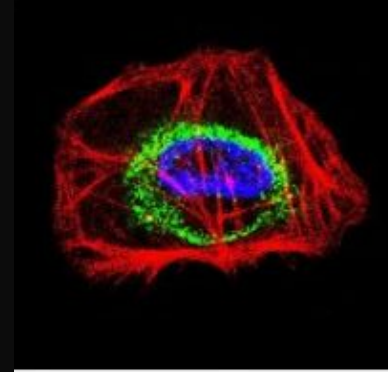
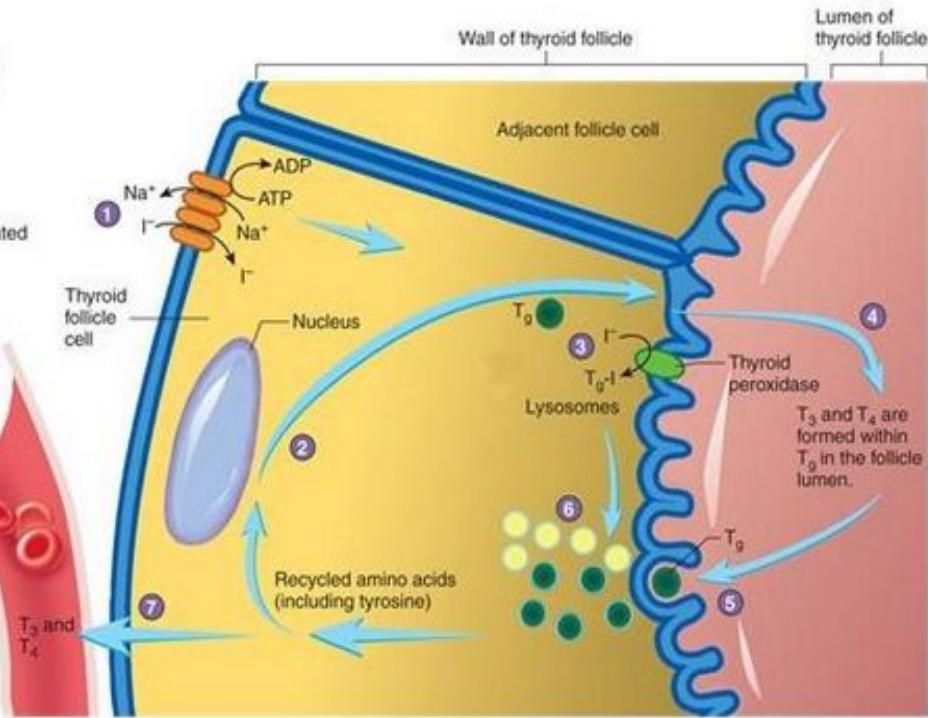
C. Toxic mng



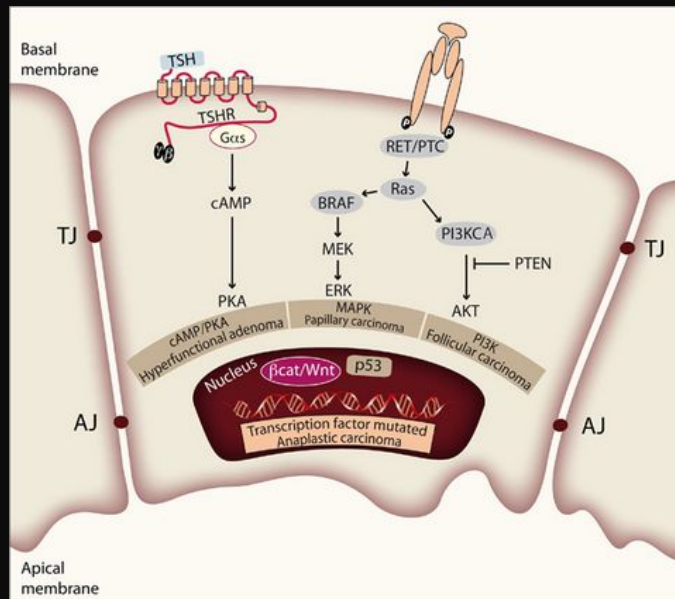
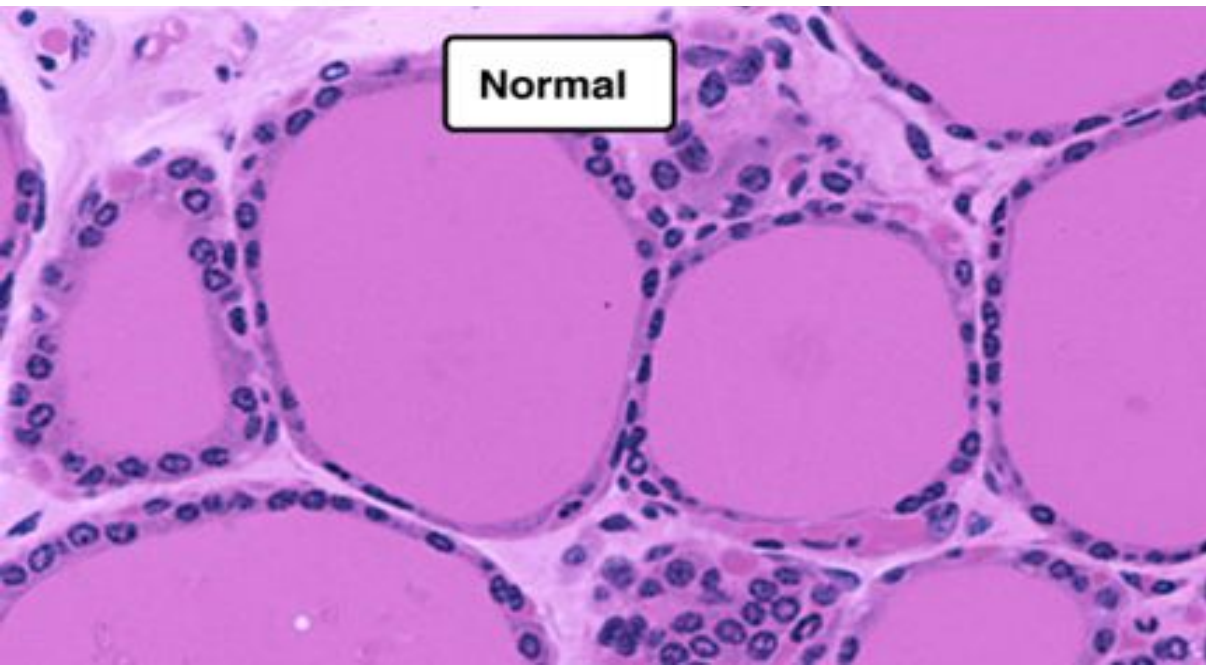
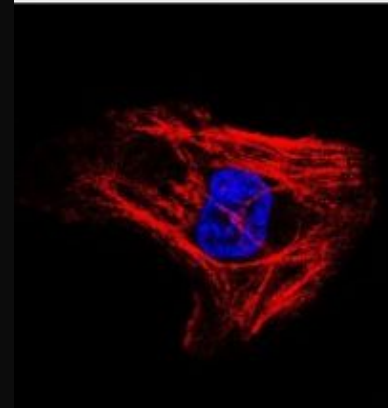
D. Toxic adenoma



- 1 Iodide is actively transported into thyroid follicle cells by a Na^+/I^- symporter.
- 2 Thyroglobulin (T_g) is synthesized in the thyroid follicle cell.
- 3 Tyrosines within T_g are iodinated by thyroid peroxidase.
- 4 Two iodinated tyrosines within T_g join to form tetraiodothyronine (T_4) or triiodothyronine (T_3).
- 5 Endocytosis of T_g into the thyroid follicle cells.
- 6 T_g is digested by lysosomal enzymes into individual amino acids and T_3 and T_4 . The amino acids are recycled into thyroglobulin.
- 7 T_3 and T_4 diffuse out of the thyroid follicle and enter the circulatory system.

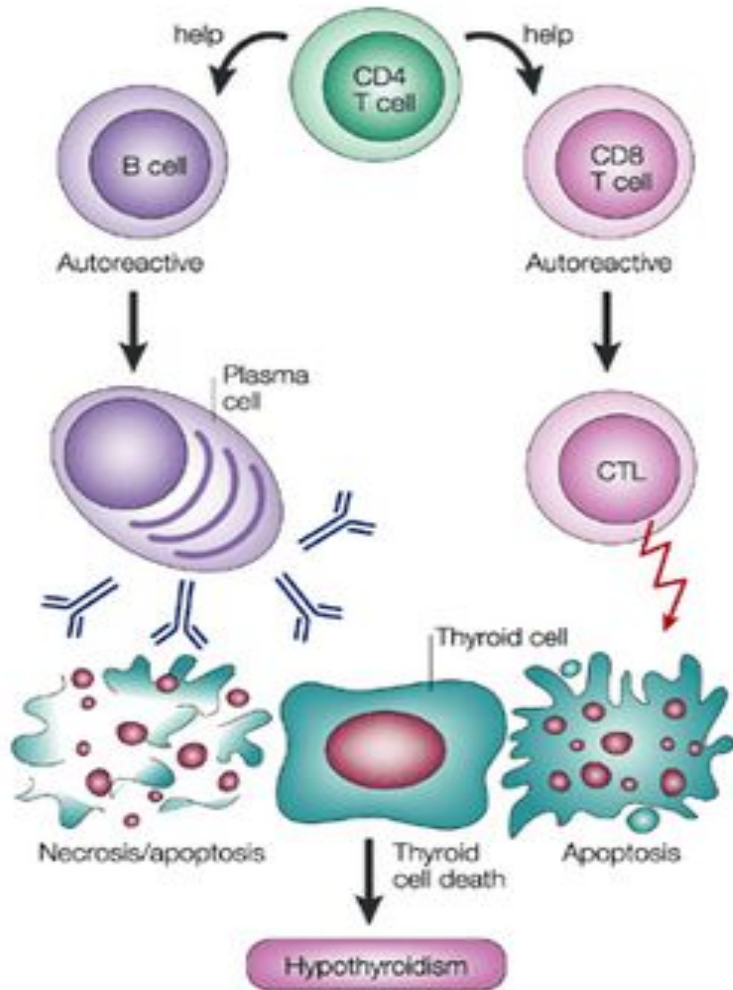


TSHR
In vitro

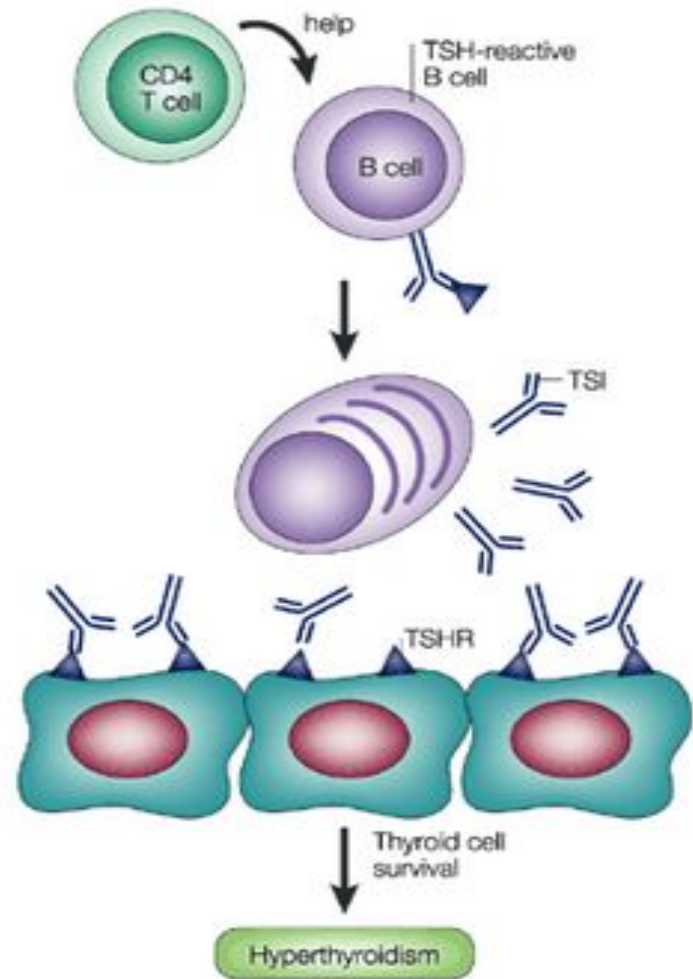


Fisiopatogenia

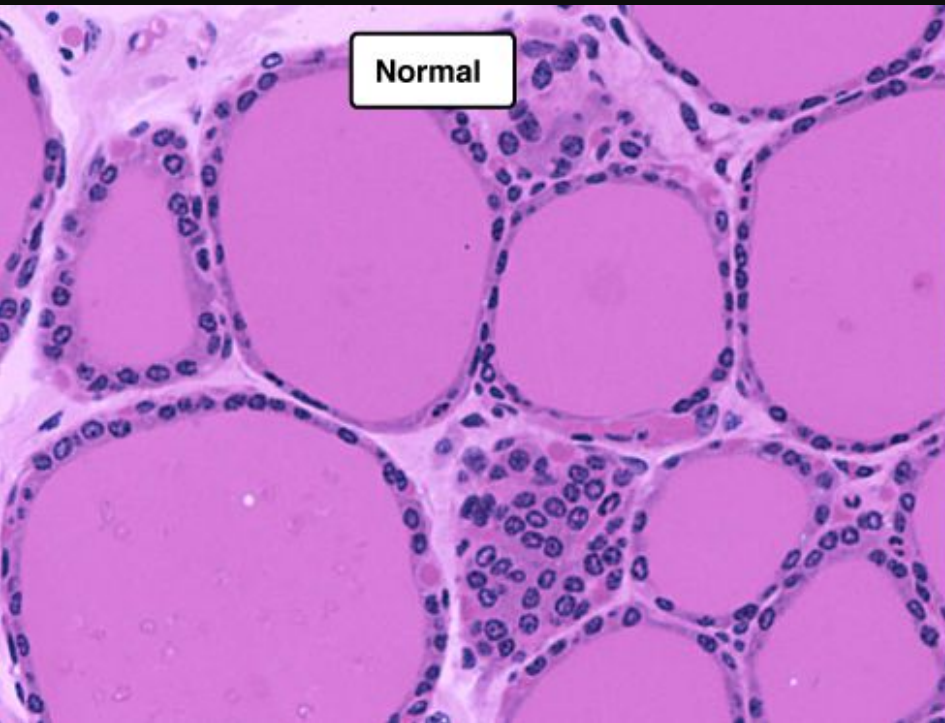
a Hashimoto's thyroiditis



b Graves' disease



Histopatologia



Paciente



Hipertireoideo

Magro

Bócio

Exoftalmia

Ansioso

Distúrbios do sono

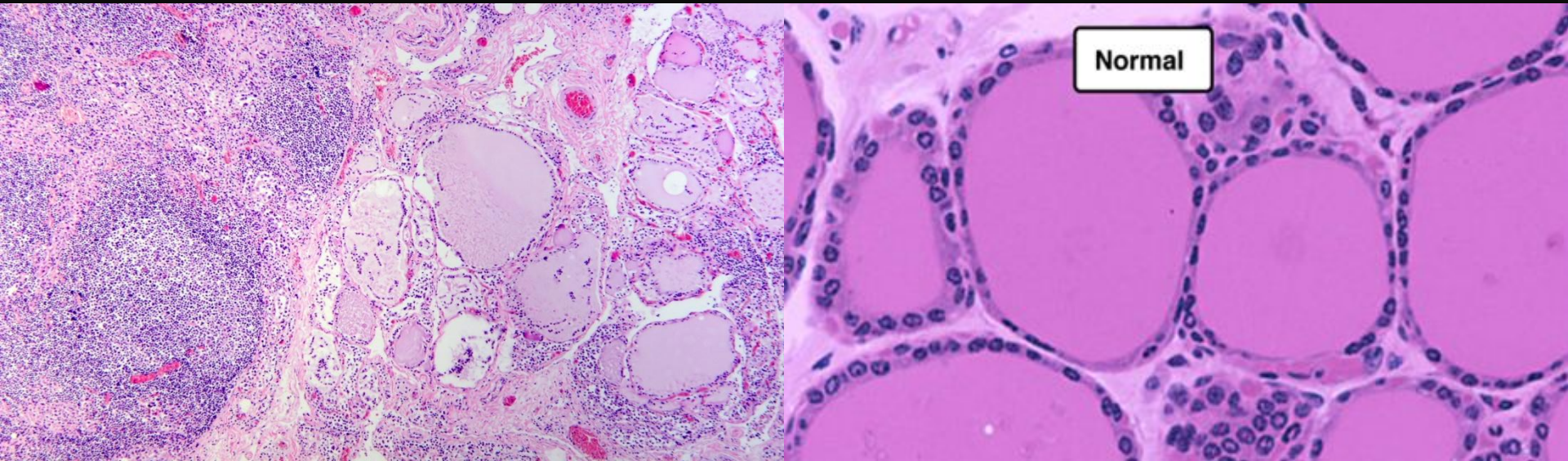
Metabolismo acelerado



Tireoidite de Hashimoto – Tireoidite Crônica Linfocítica

- 3,5 – 1000 mulheres – 14 / 1000 após 60 anos de idade
- Comum doença se iniciar após a puberdade ou gravidez
- HLA – DR3, HLA – DR4, HLA – DR5
- Antígenos
 - Anti-tireoglobulina – 100% pacientes
 - Anti-TPO (tireoperoxidase)
 - Anti-TSH – 10-20% pacientes (bloqueador)
- Infiltrado de células Th1 – IFN- γ
- Células T CD8 citotóxicas também estão presentes

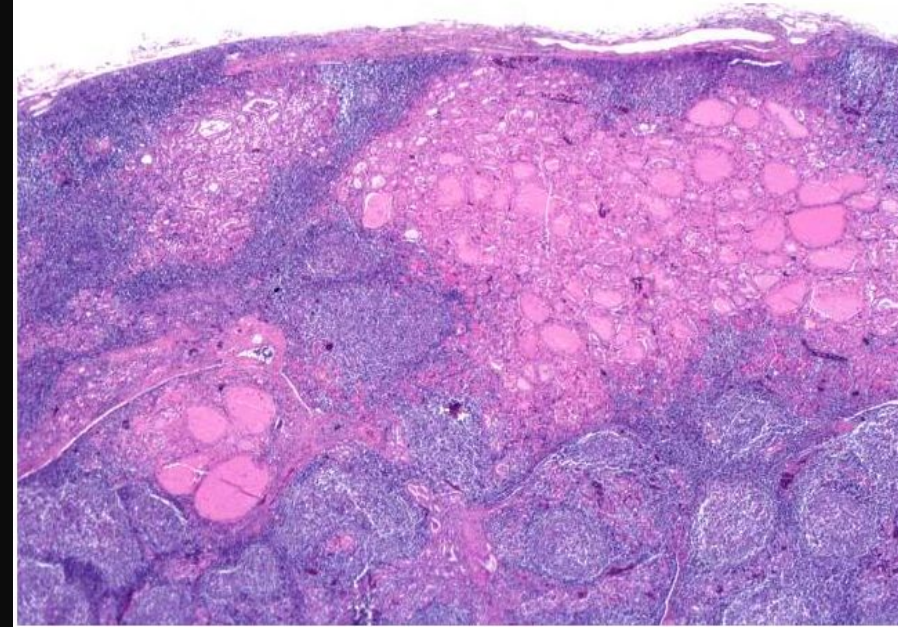
Histopatologia



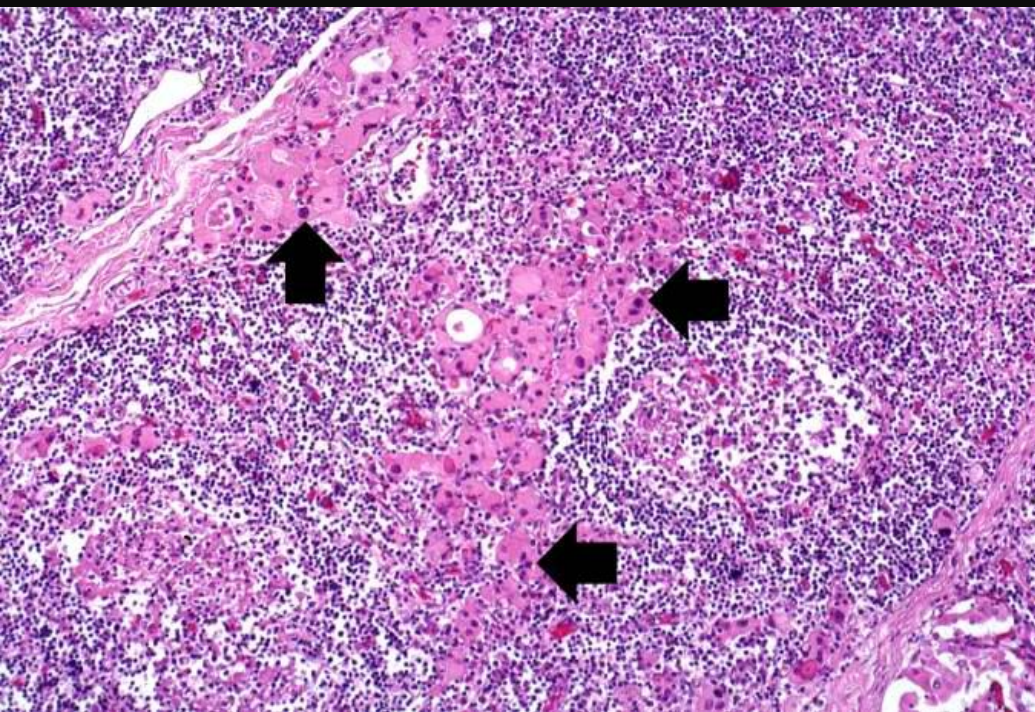
Hipotireoideo
Metabolismo Lento
Obeso



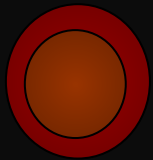
Hurtle Cells
Células foliculares em degeneração
Marcação eosinofílica
Iniciar nódulos tumorais



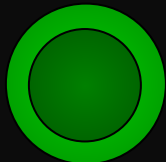
Formação de Centros Germinativos



Células Imunes Infiltrantes do SNC são Alvos de Neurotransmissores



CD4



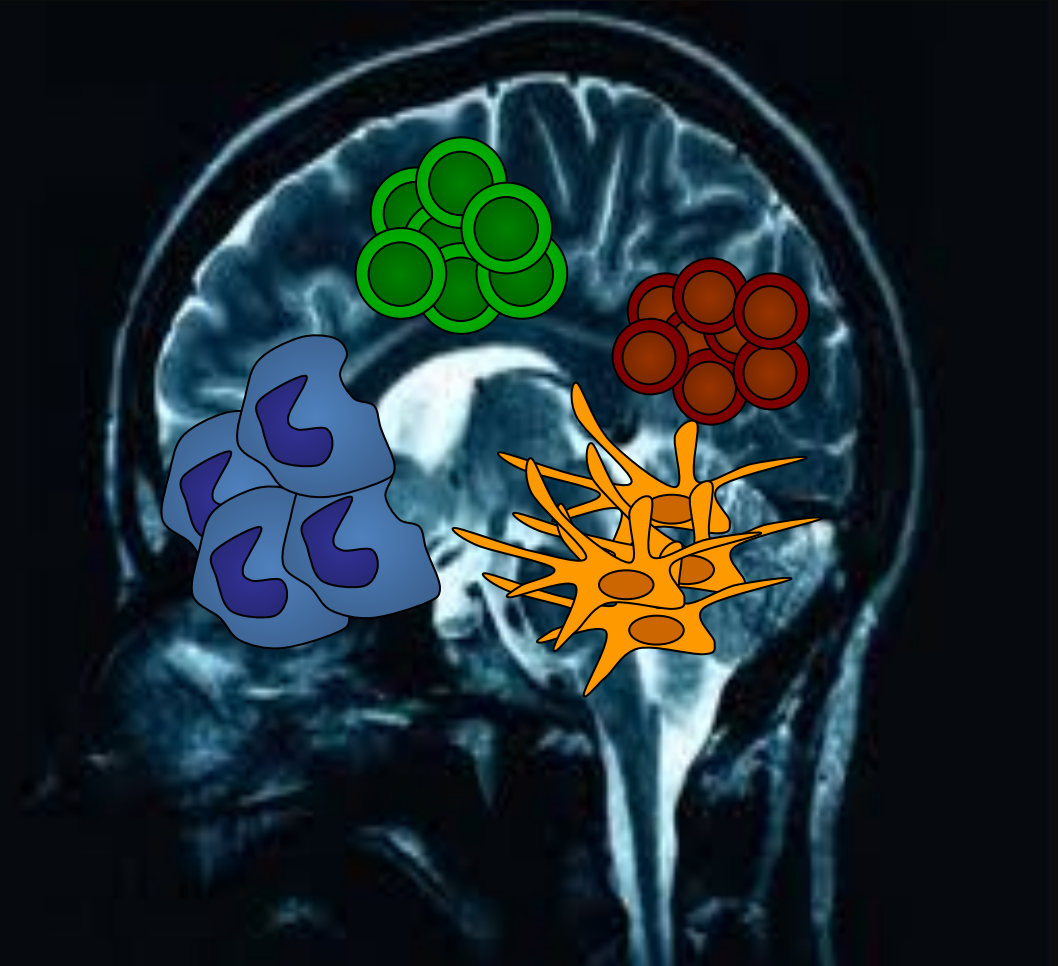
CD8



Mφ



DCs



Encefalomielite Experimental Auto-imune EAE

Modelo Murino para Estudo da Esclerose Múltipla

Ativação do Sistema Imune

Características

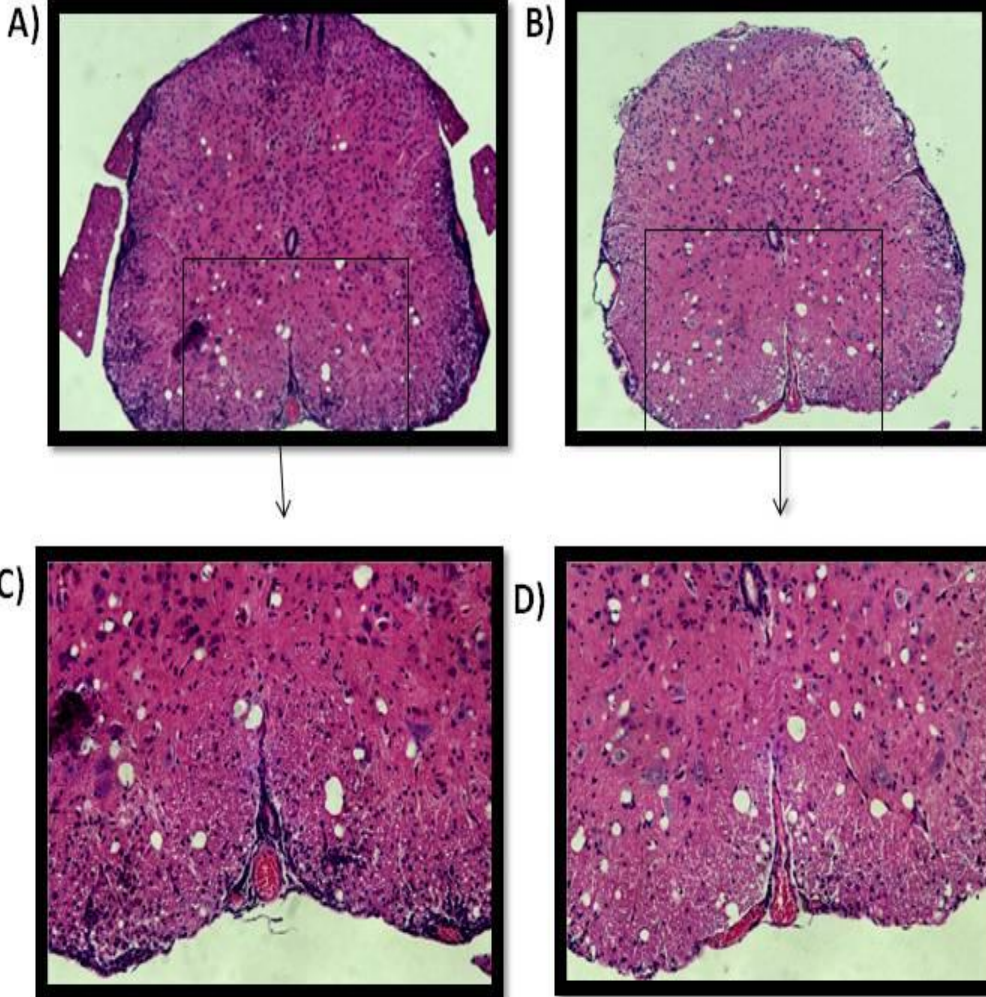
Infiltrado inflamatório perivascular
Gliose, Desmielinização,
Lesão axonal e morte neuronal

Utilizando este Modelo
4 Linhas de Pesquisa Principais

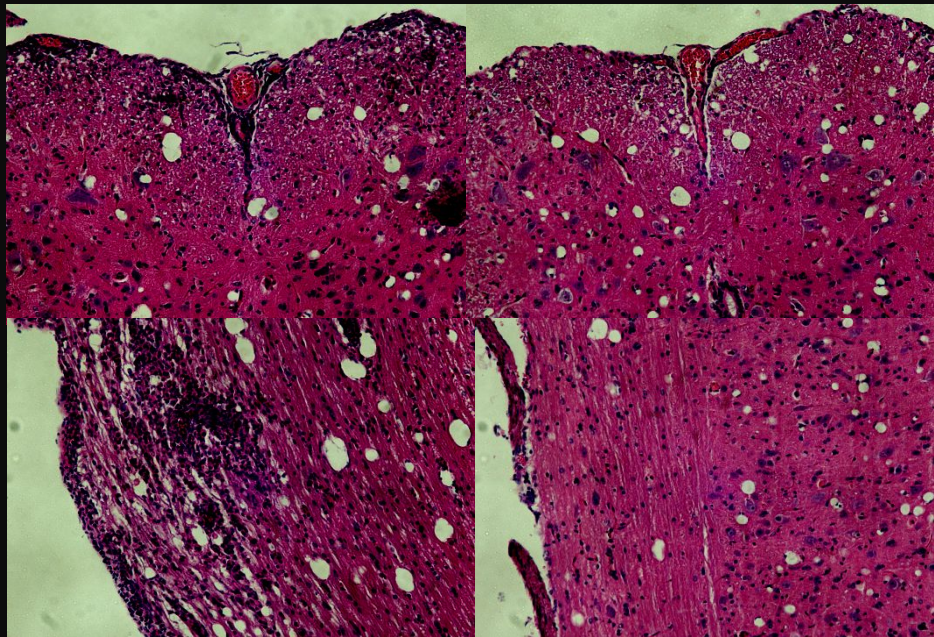
- 1) Tolerância Oral
- 2) Papel das Células da Microglia
- 3) Tratamento com MSCs Humanas
- 4) Papel do neurotransmissor Glutamato como imunomodulador.

PBS

MOG



Encefalomielite Experimental Auto-imune



Modelo Murino para Estudo da Esclerose Múltipla

Características

Infiltrado inflamatório

Macrófagos, T CD4, TCD8, DCs

Ativação das Células Residentes

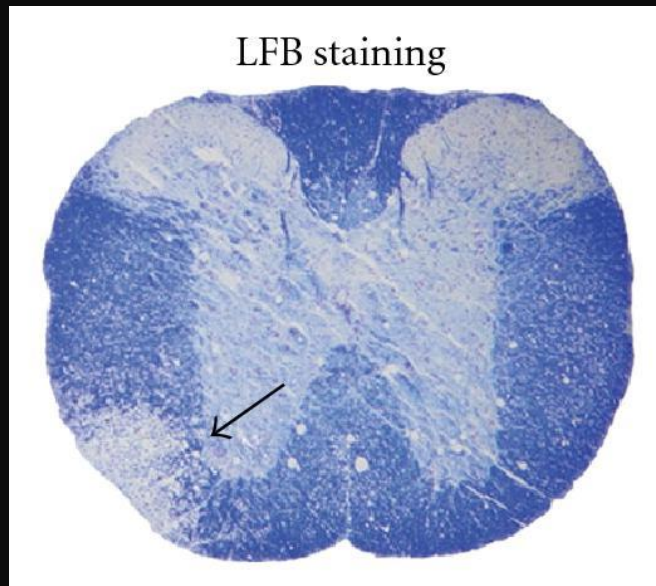
Gliose, Ativação de Células da Microglia

Desmielinização,

Lesão Axonal e Morte Neuronal

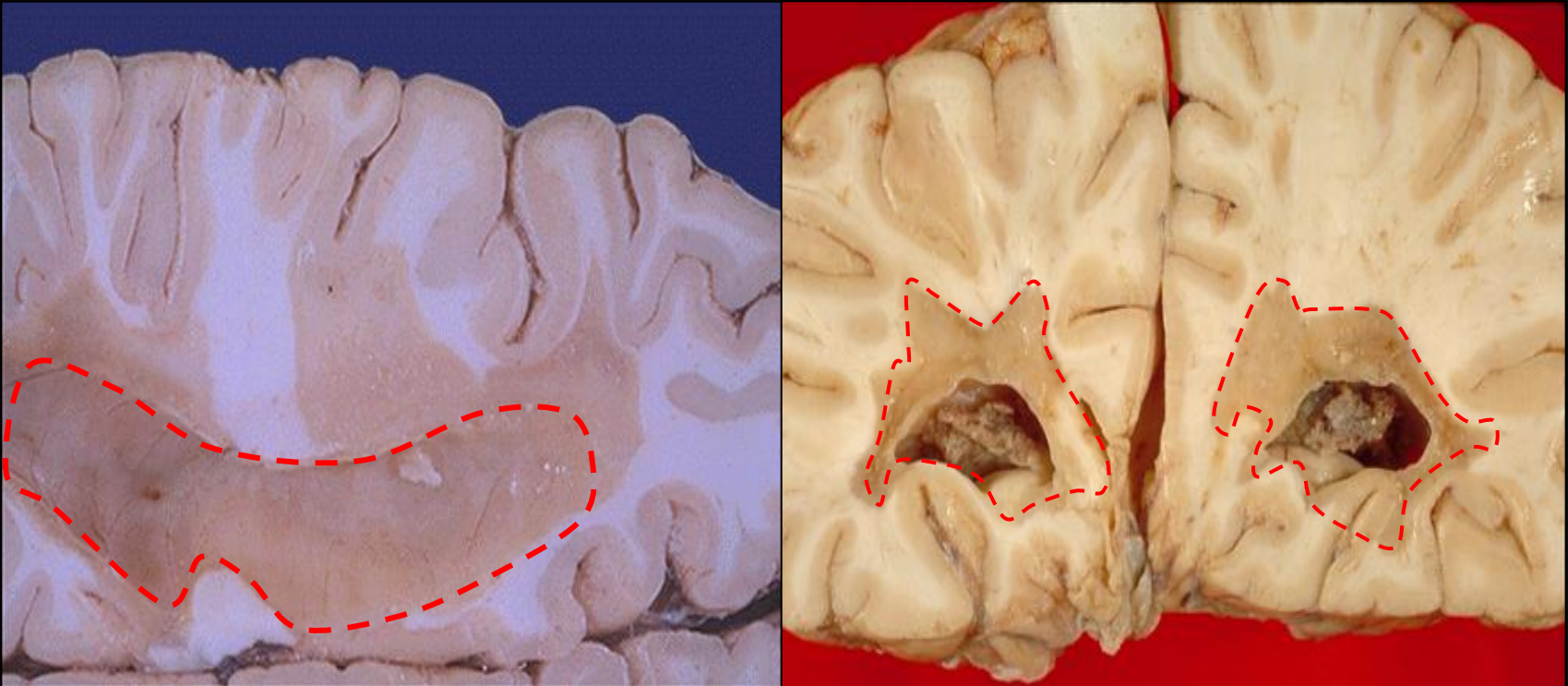
Utilizando este Modelo

- 1) Papel das Células da Microglia
- 2) Tratamento com MSCs Humanas
- 3) Papel do neurotransmissor Glutamato como imunomodulador.



Lesões no Sistema Nervoso Central

- Placas Peri-ventriculares (Desmielinização)

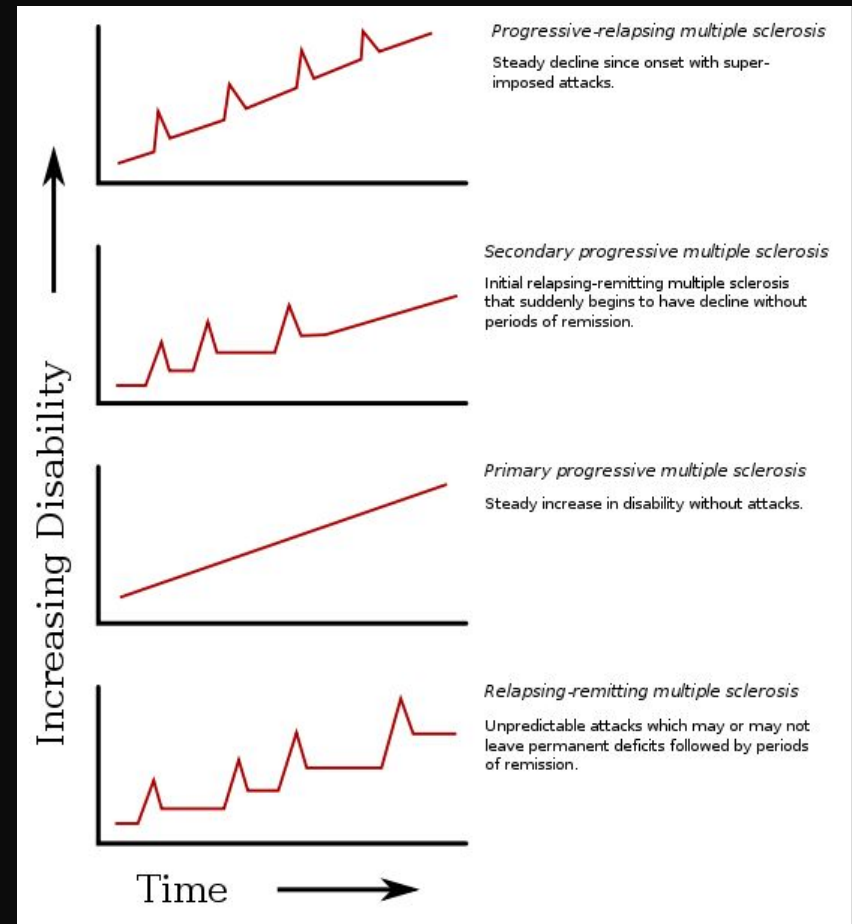


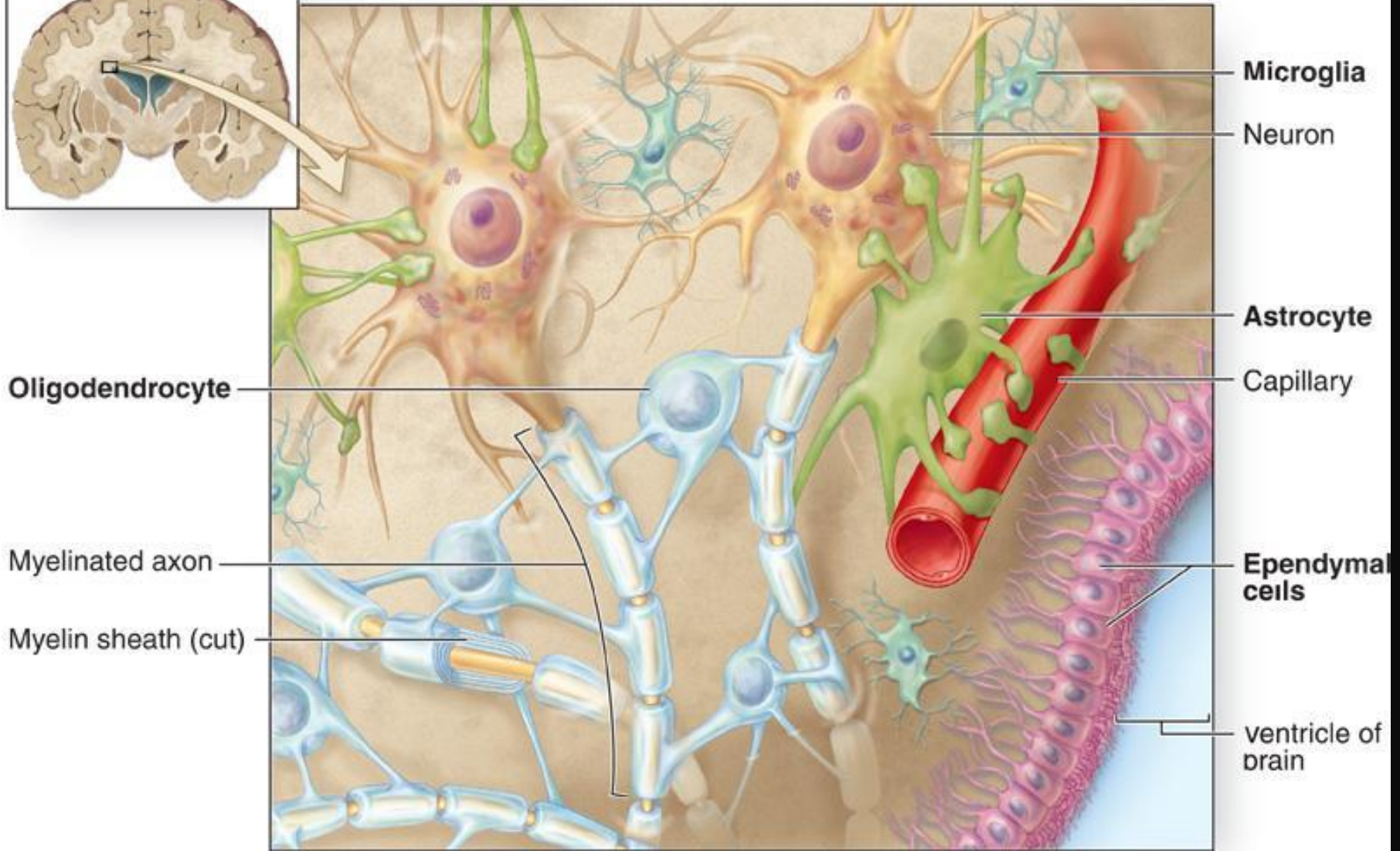
Camundongos com EAE



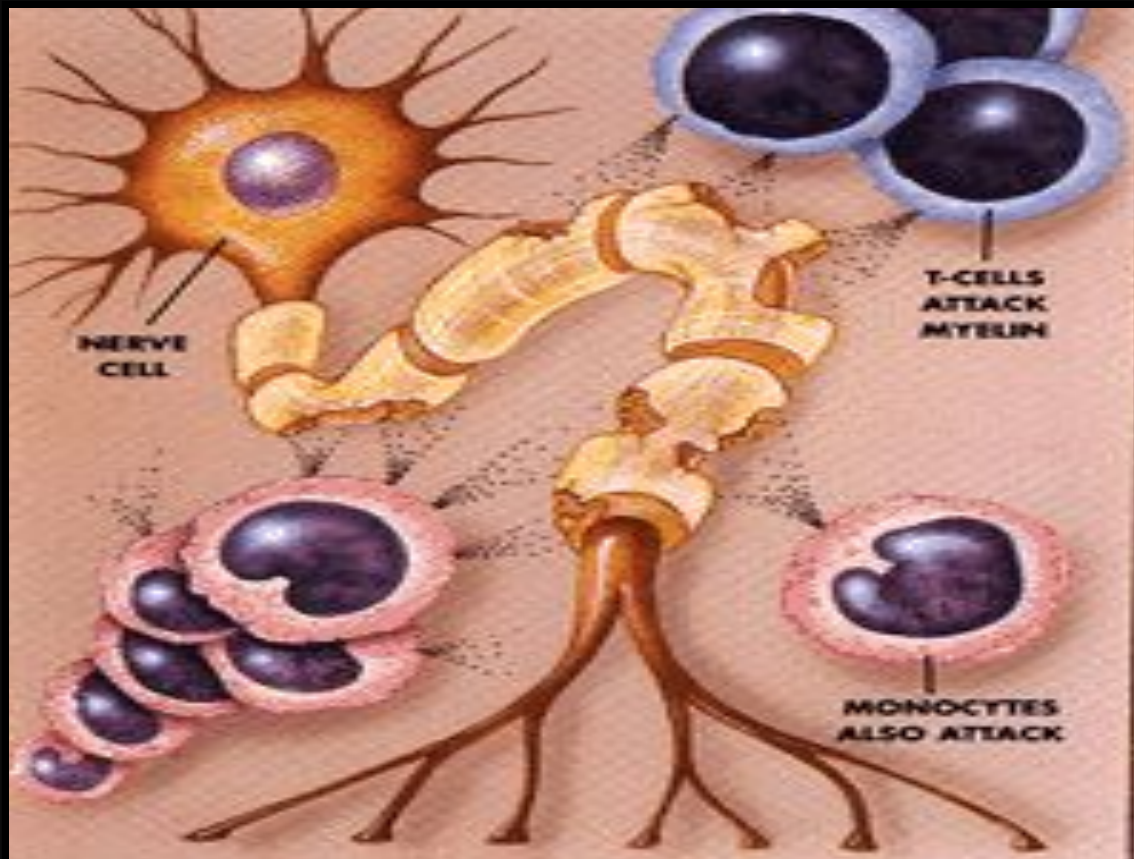
Esclerose Múltipla

- Inflamação do SNC
 - Infiltrado Inflamatório
 - Gliose
 - Desmielinização
 - Morte neuronal
 - Lesões predominantemente perivasculares
- Progressiva e Remissão
- Secundária progressiva
- Primária progressiva
- Pico e remissão





O Papel do Sistema Imune



Citocinas

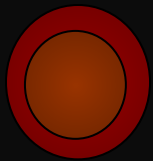
IL-1, IL-6, IL-12
IL-17, IL-23, IFN-
Y

NO
ROIs

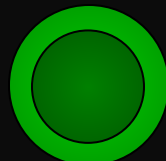
MMP-3
MMP-9

Granzimas

IDO
Glutamato



CD4



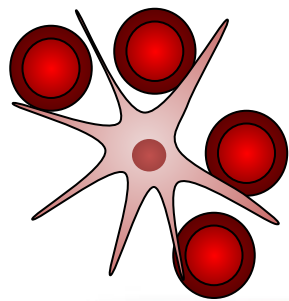
CD8



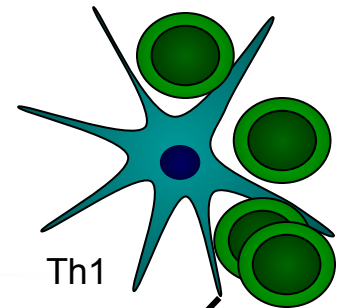
Mφ



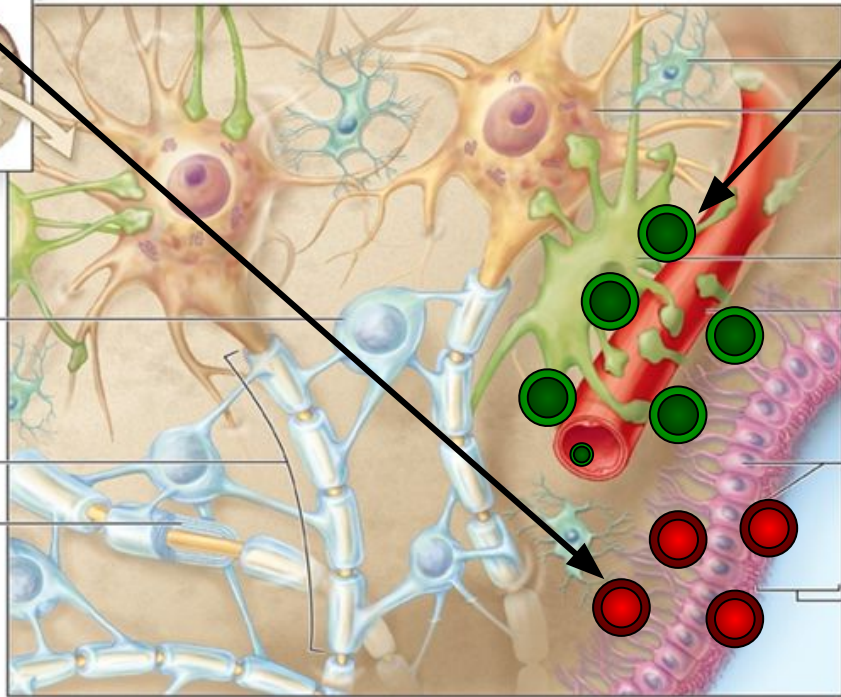
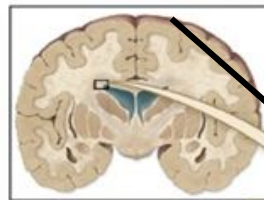
Células Dendríticas



Th17



Th1



Oligodendrocyte

Myelinated axon

Myelin sheath (cut)

Microglia

Neuron

Astrocyte

Capillary

Ependyma cells

ventricle of brain

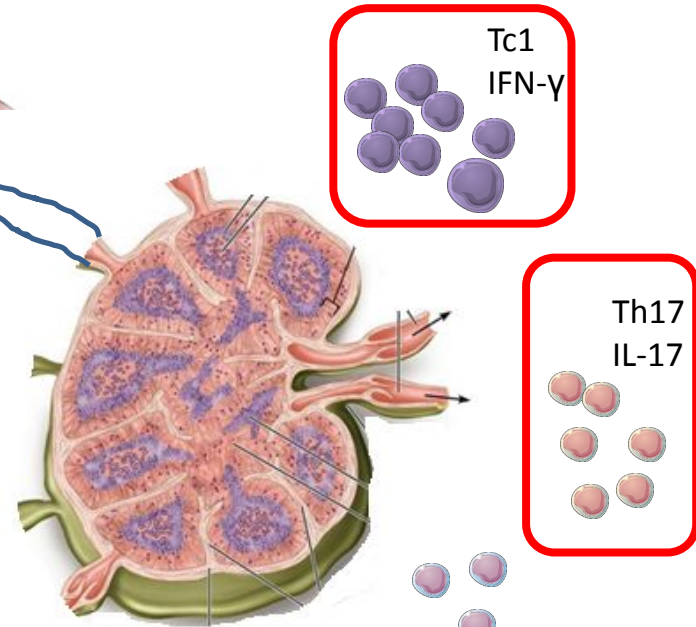
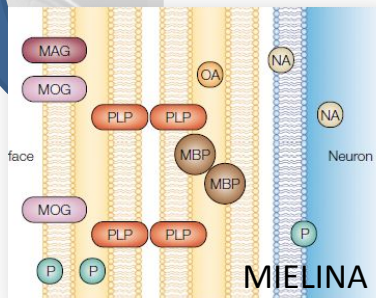
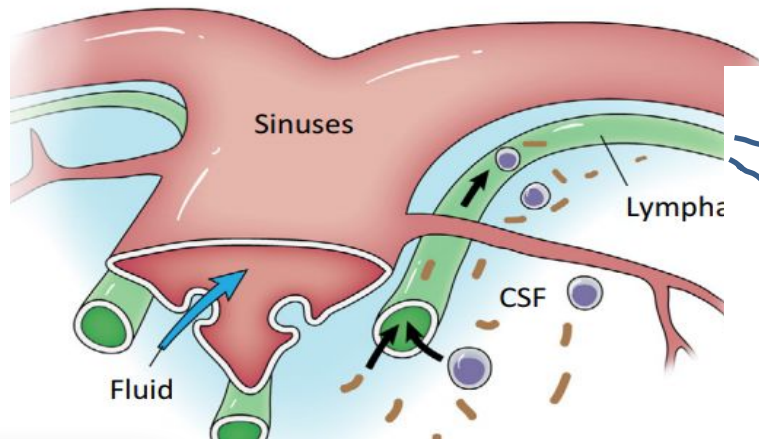
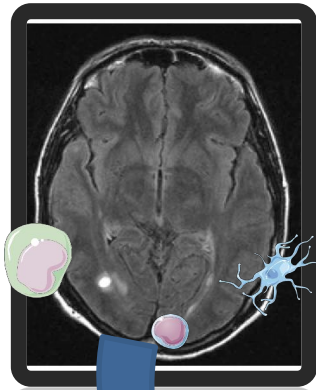
Rothhammer V. et al.
Th1 - VLA-4

Reboldi A. et al 2008
CCR6 - CCL21

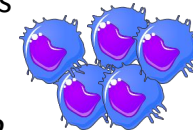
Murphy AC 2010

Bloqueio da Infiltração do SNC - Imunovigilância

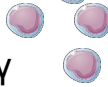
2/3 apresentam progressão

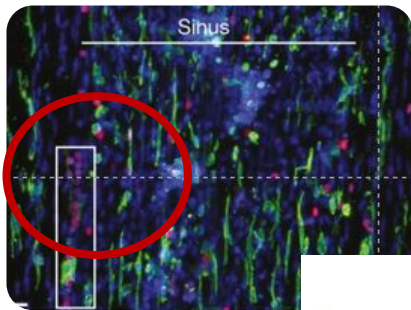


Macs
IL-1
IL-6
IL-12



Th1
IFN-γ

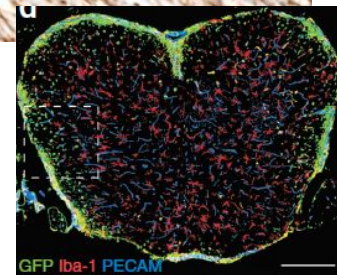
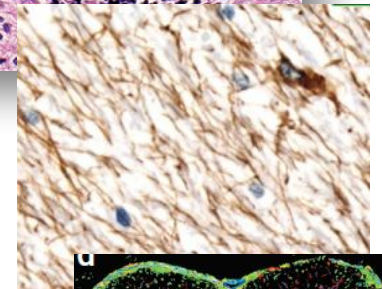
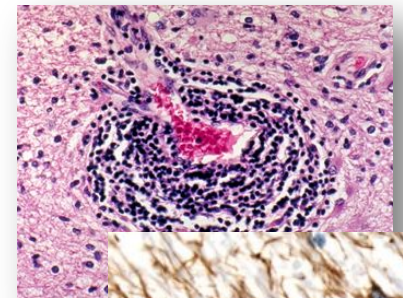
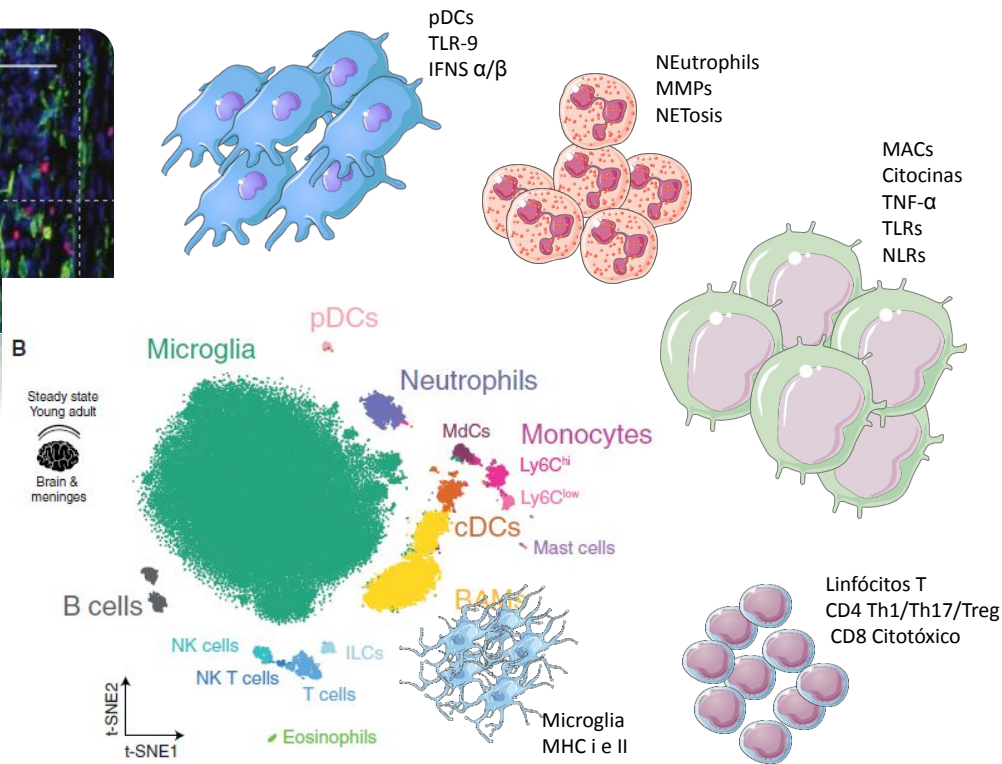




Cytox

Citometria de Fluxo
 Associada à
 Espectrometria de
 Massa
 44 – Parâmetros

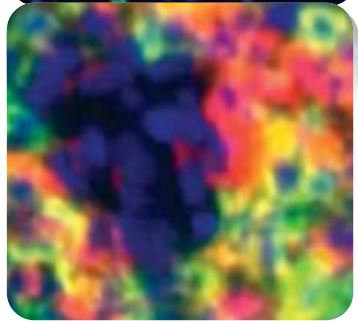
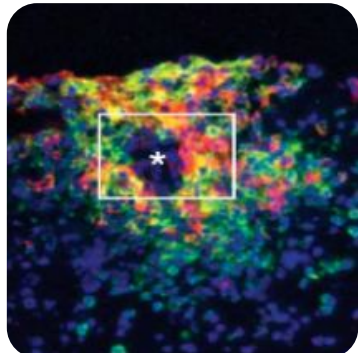
Citometria de Luz
 22 Parâmetros





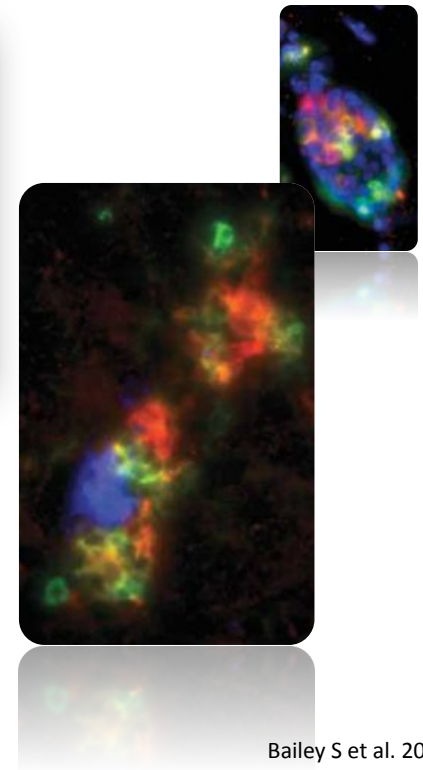
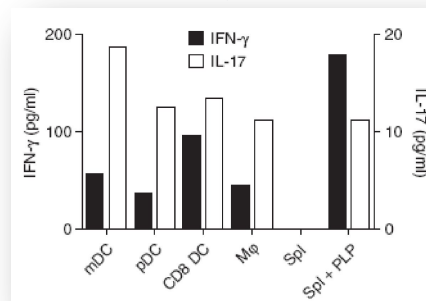
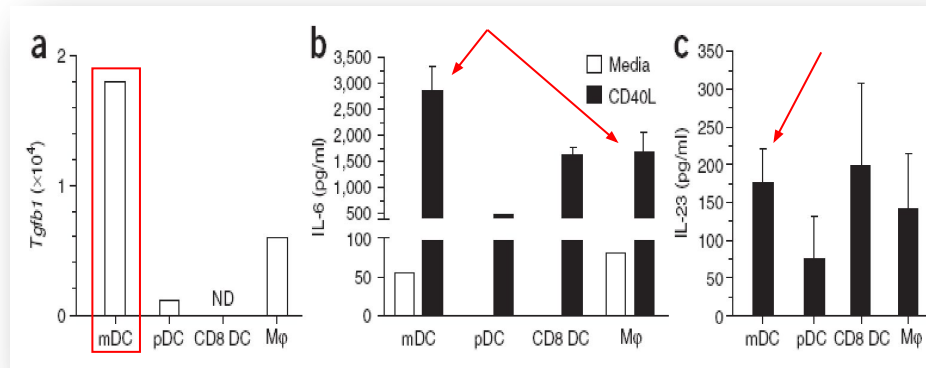
Mas...
Quem ativa os linfócitos
Infiltrantes ?

Epitope Spreading?
Bystander Activation?

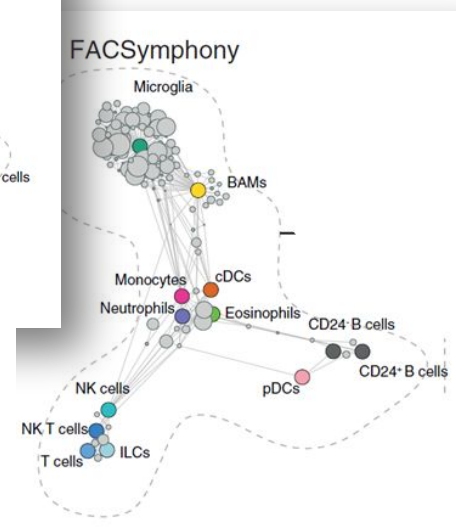
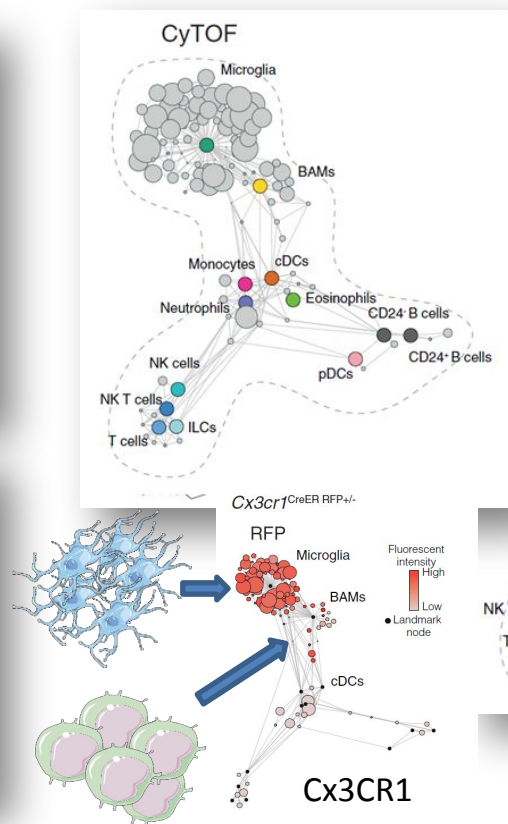
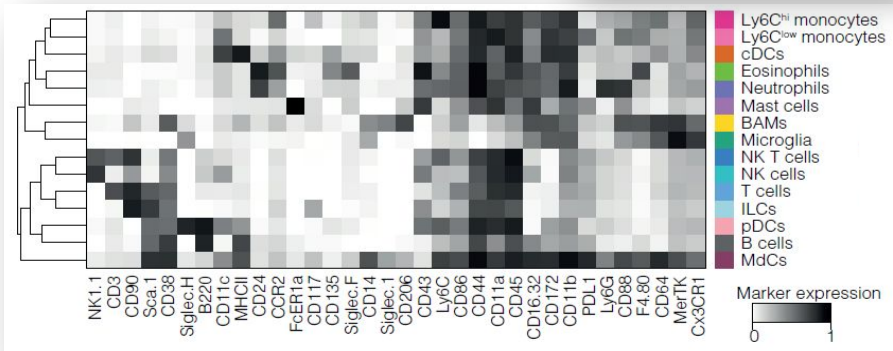
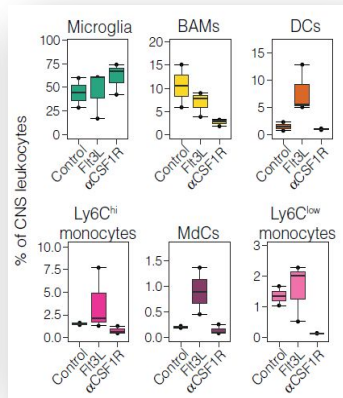
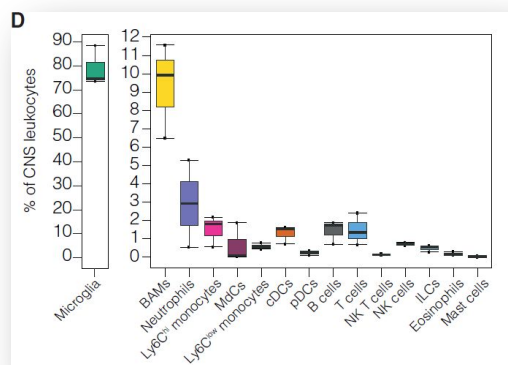


Red: CD11c
 Yellow: CD11b
 Blue: Thy1.1

Co-Cultura – Células Dendríticas do Cérebro + Linfócitos T

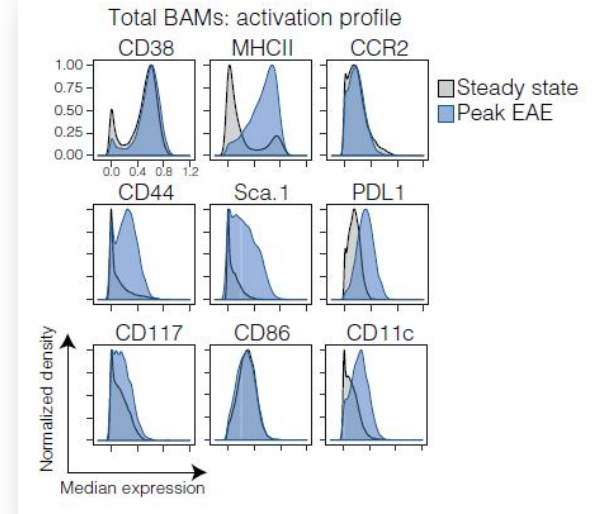
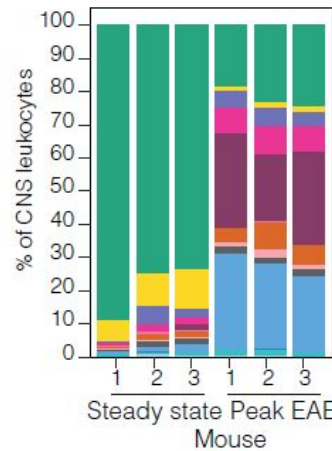
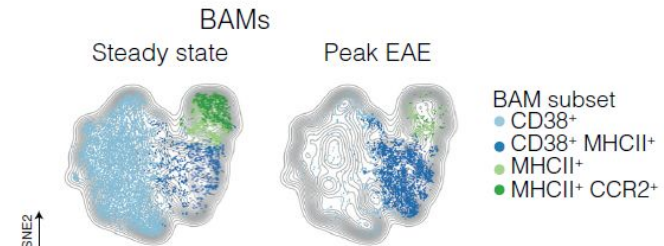
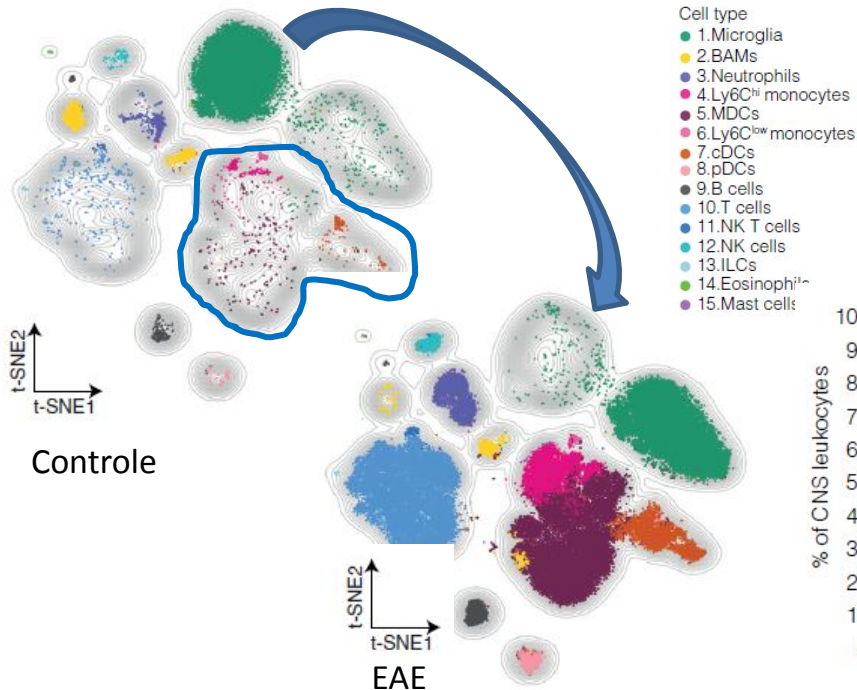
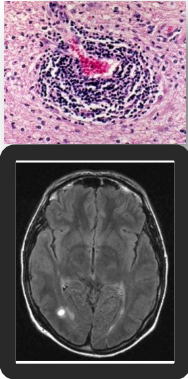


Macrófagos, Células Dendríticas, Monócitos e Neutrófilos Fazem Parte do Pool de Células Associadas ao SNC

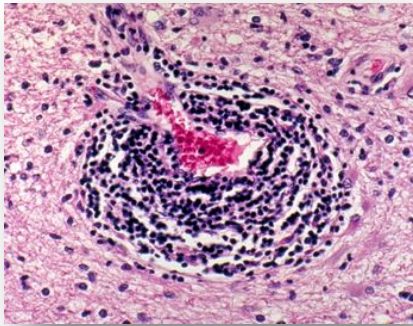


Como Se Comporta Cada População Durante a Neuroinflamação?

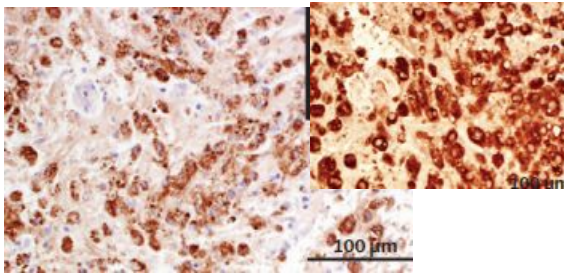
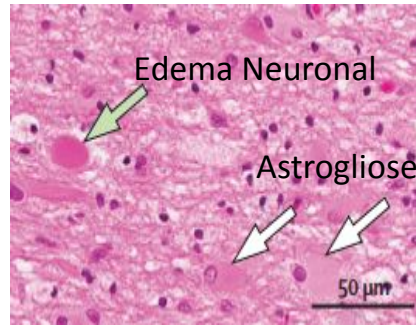
Qual Sua Relevância Para a Neuroinflamação?



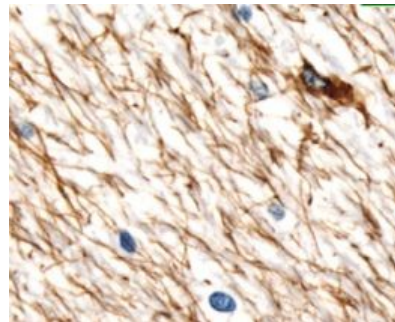
Histopatologia da MS



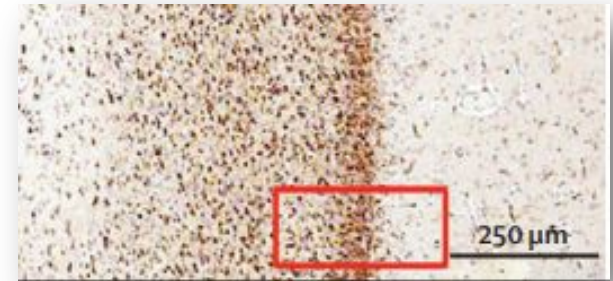
Infiltrado perivascular



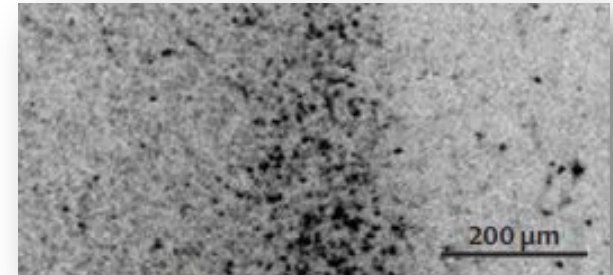
Macrófagos PLP⁺



GFAP⁺



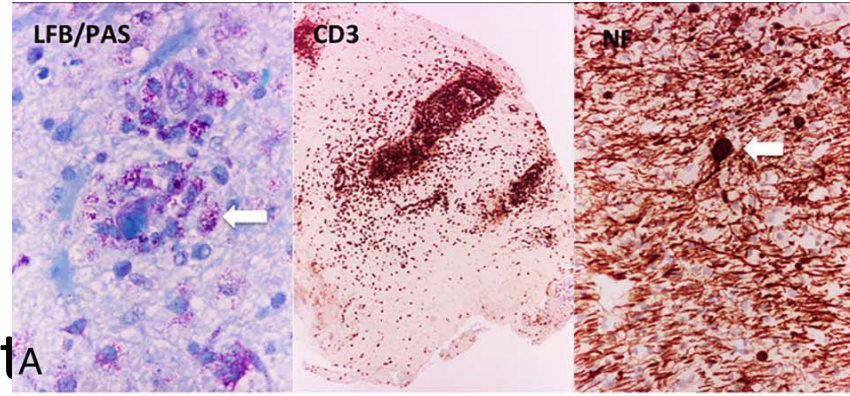
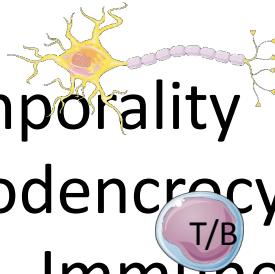
Placa de Macrófagos



Macrófagos Ferro⁺

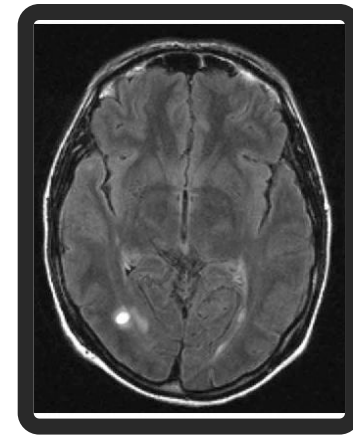
Multiple Sclerosis

- Chronic Disease - Temporality
- Demyelinating – Oligodendrocyte
- Neuroinflammation - Immune System
- Neurodegenerative – Nervous System
- Autoimmune – Adaptive
- Environment and Genetic Factors
 - HLA-DR* 15:01, EBV, HERVs.
 - Smoking, microbiota, Vitamin D
- Primary Progressive
- Relapse Remitting
- Secondary Progressive



LFB/PAS (left), CD3 (middle), NF (right)

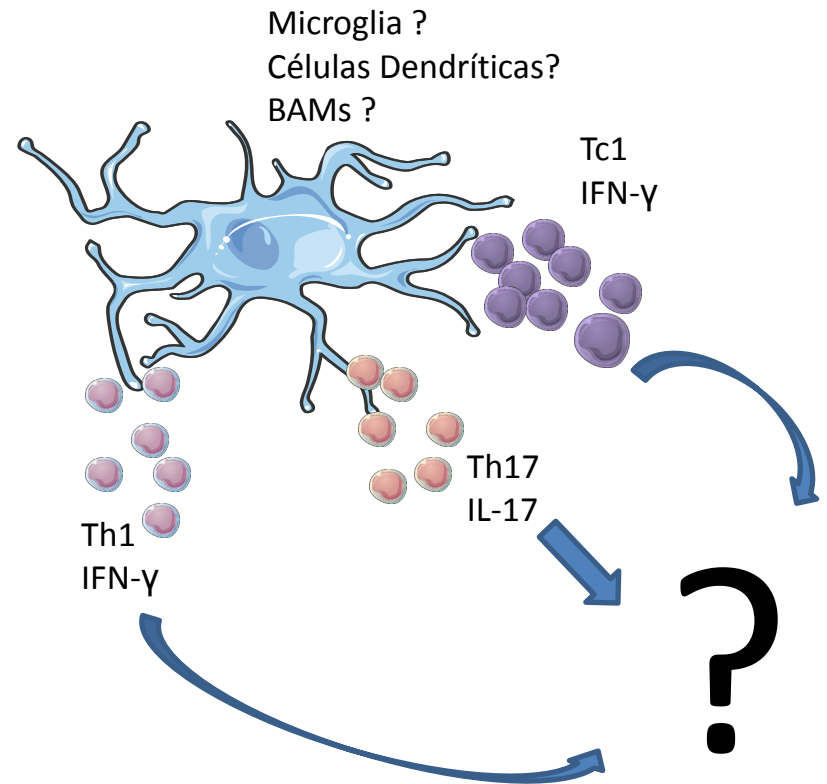
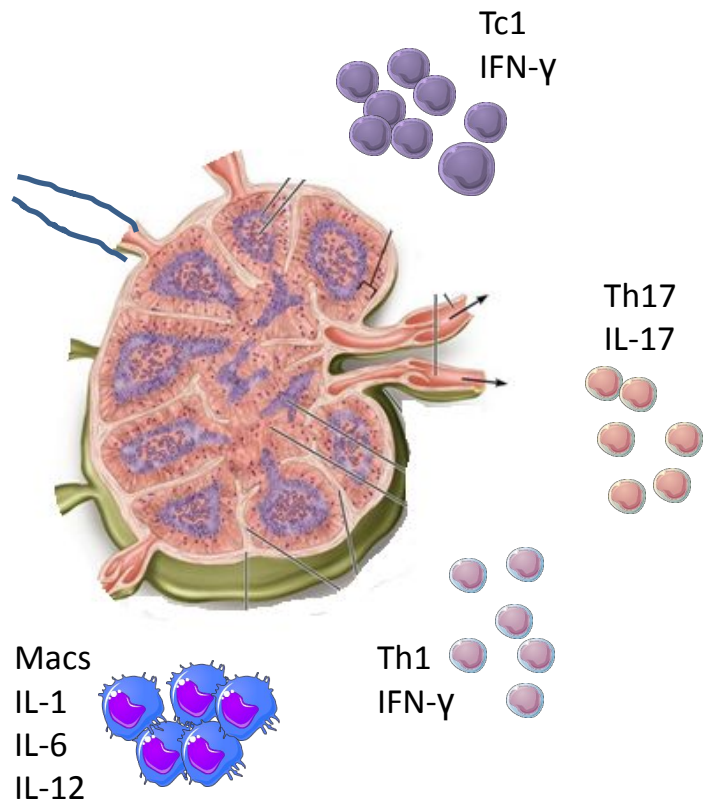
Coloração para NF



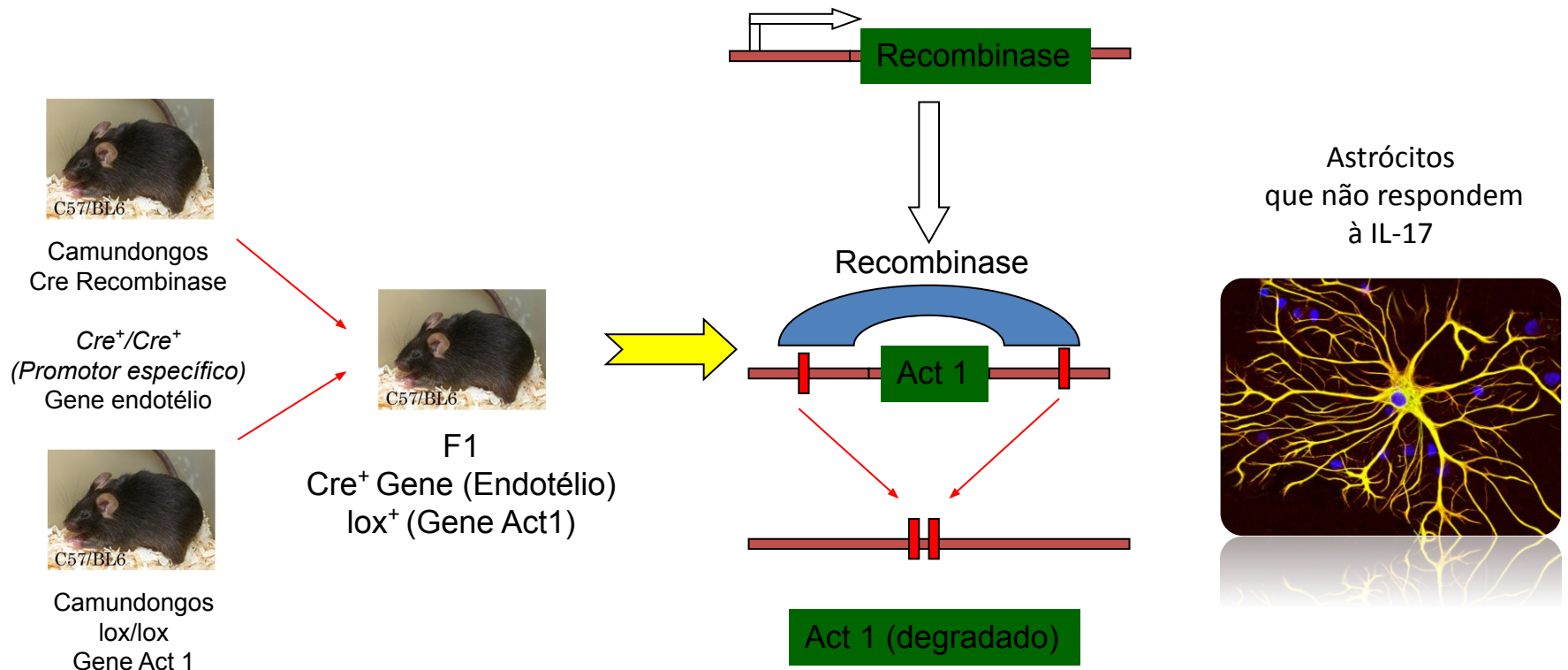
Matthews PM, Roncaroli F, Waldman A, et al 2017

Pathophysiological Differences

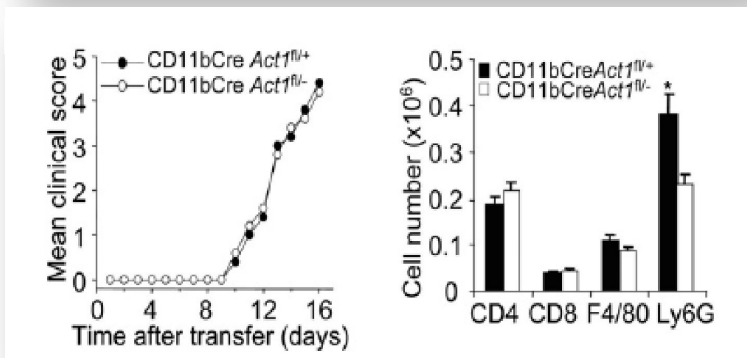
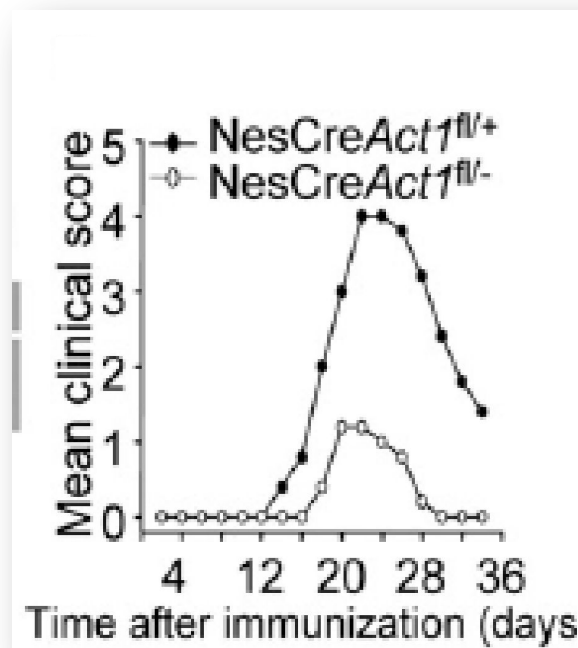
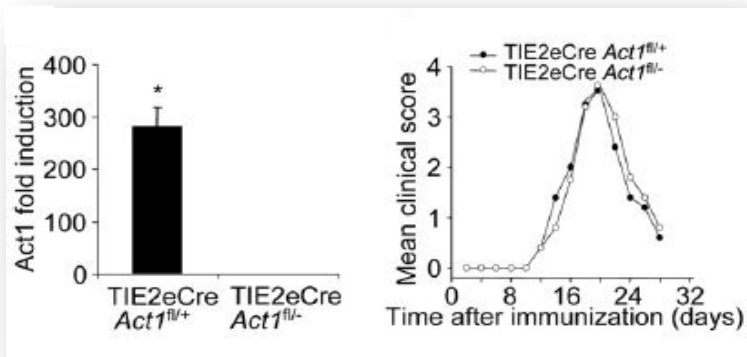
E Após a Ativação dos Linfócitos T In Situ, o Que Acontece ?



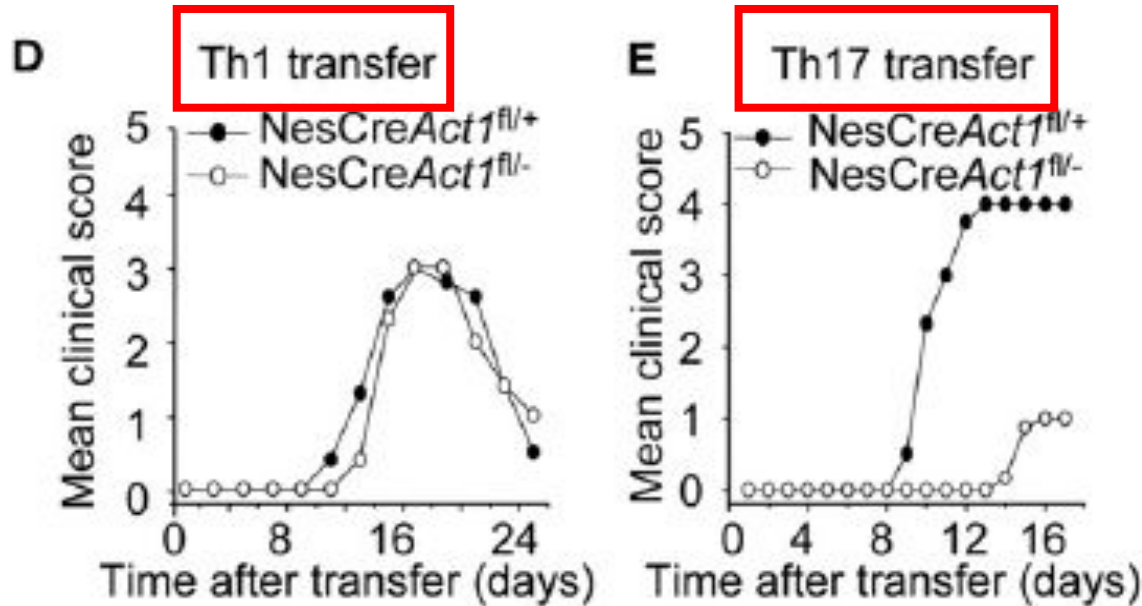
Mas, Quais os Alvos da IL-17 ? Por que ela é Tão Deletéria ?



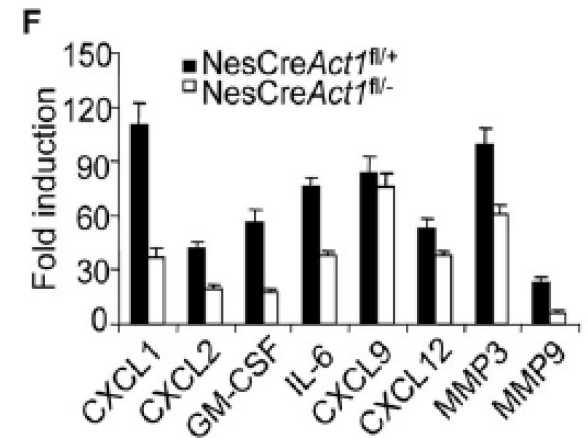
Expressão de Act1 em Endotélio e Células Mielóides é Dispensável Astrócitos São As Células Mais Responsivas à IL-17



Transferência de Linfócitos Th17 Para Animais Deficientes em do Receptor de IL-17 em Astrócitos NÃO Desenvolve

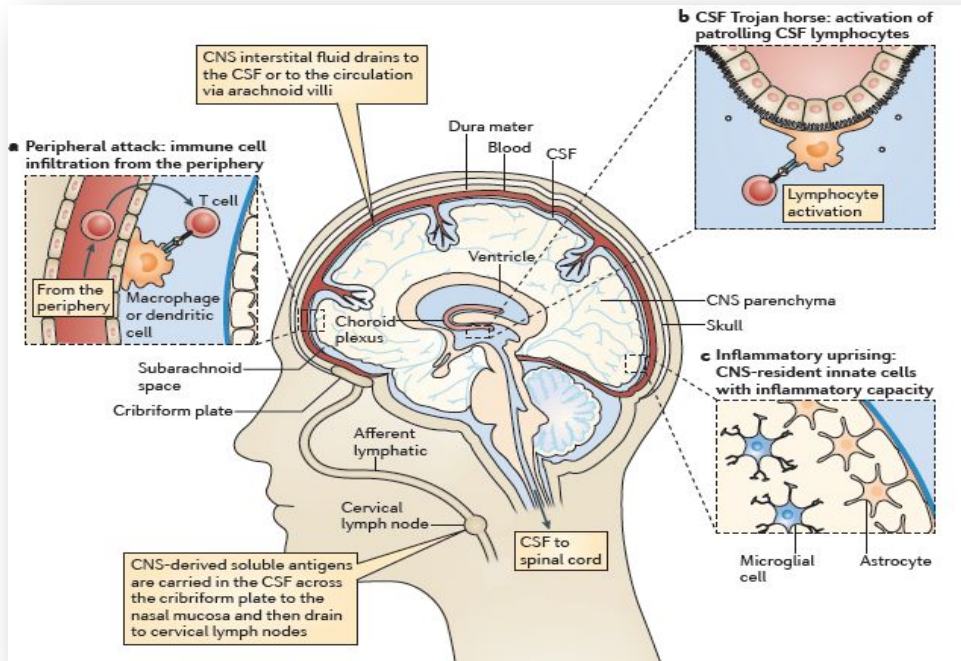


Astrócitos Estimulados In Vitro Com IL-17 Secretam Uma Série De Citocinas e Quimiocinas



Immunopathology of multiple sclerosis

Calliope A. Dendrou¹, Lars Fugger^{1,2} and Manuel A. Friese³



Second Hit

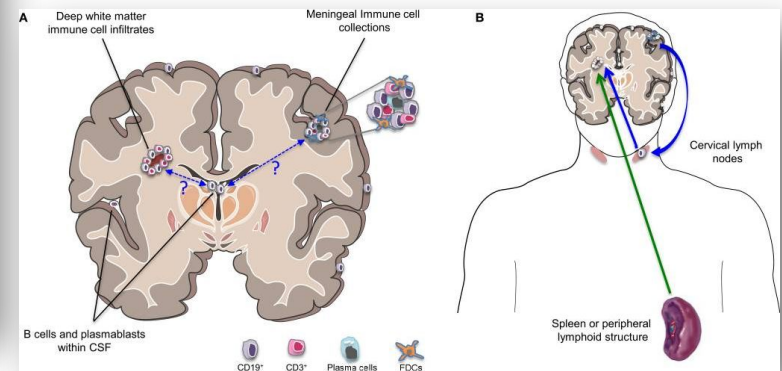
Microglia ?

Células Dendríticas SNC ?

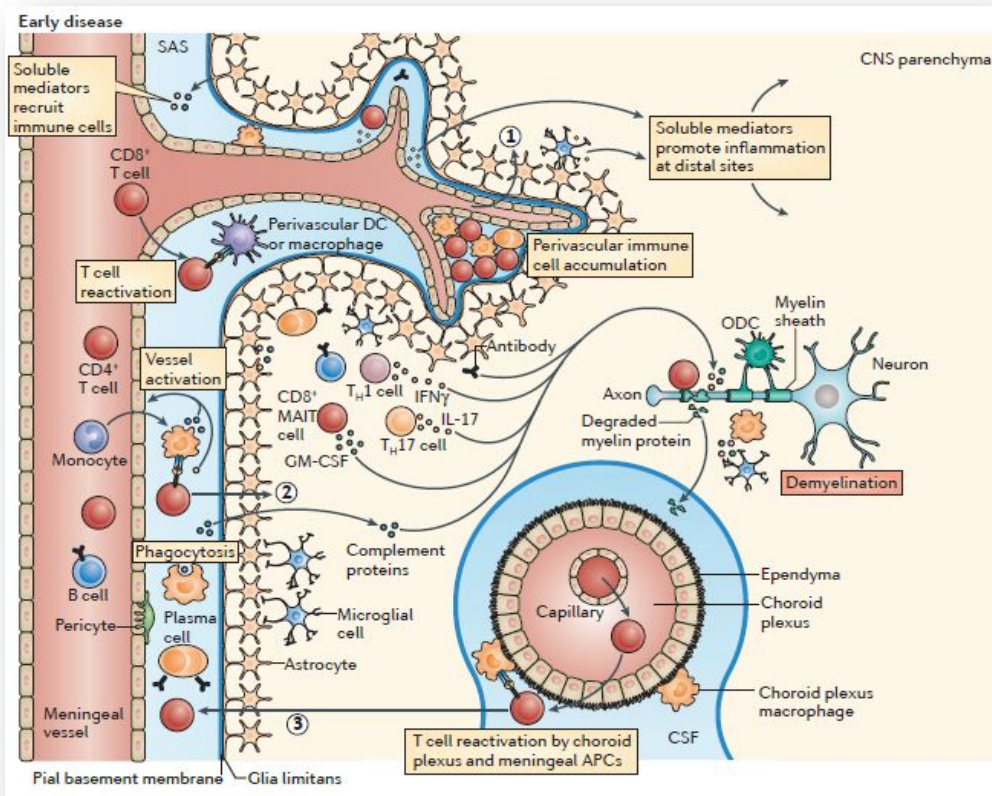
Astrócitos ?

Linfócitos B infiltrantes ?

Formação dos Folículos-like

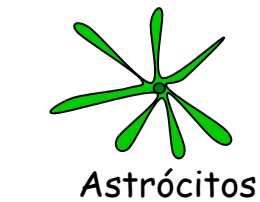
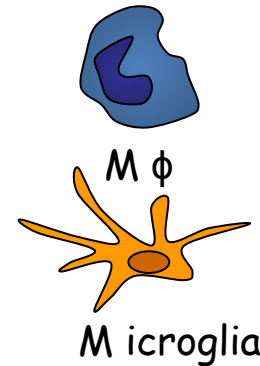
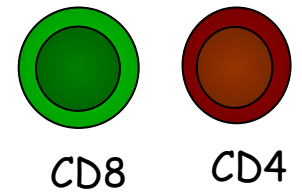


Início das Lesões – Ativação por Células Residentes

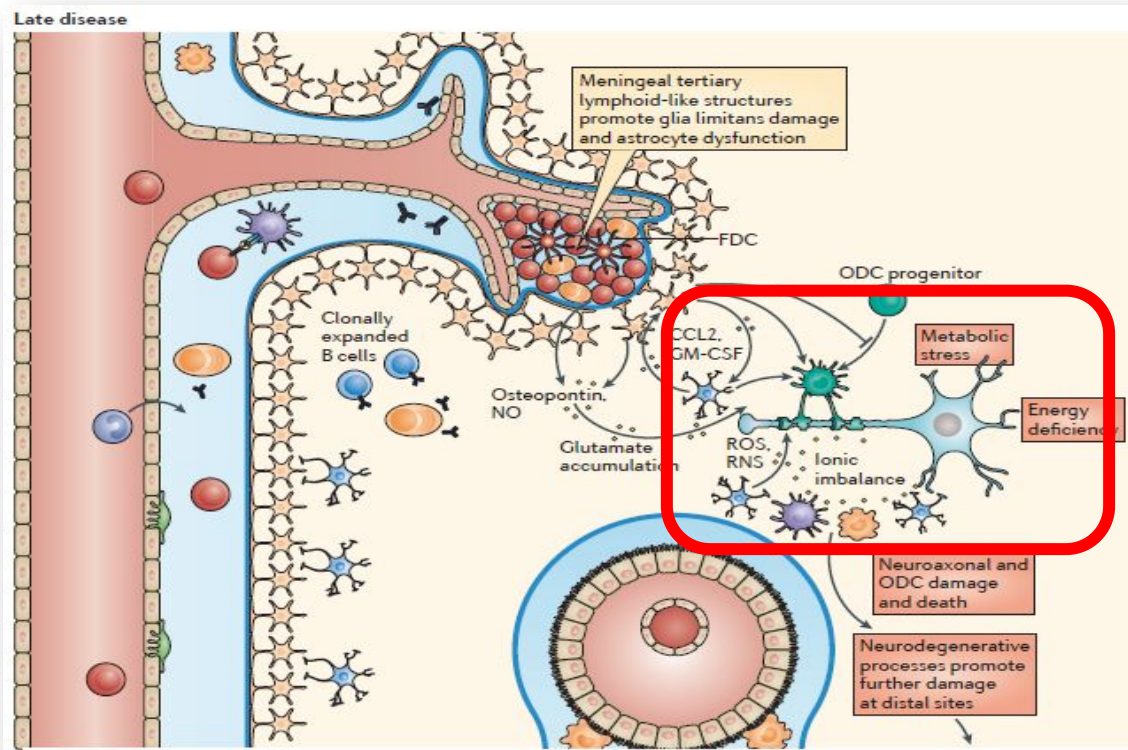


Citocinas

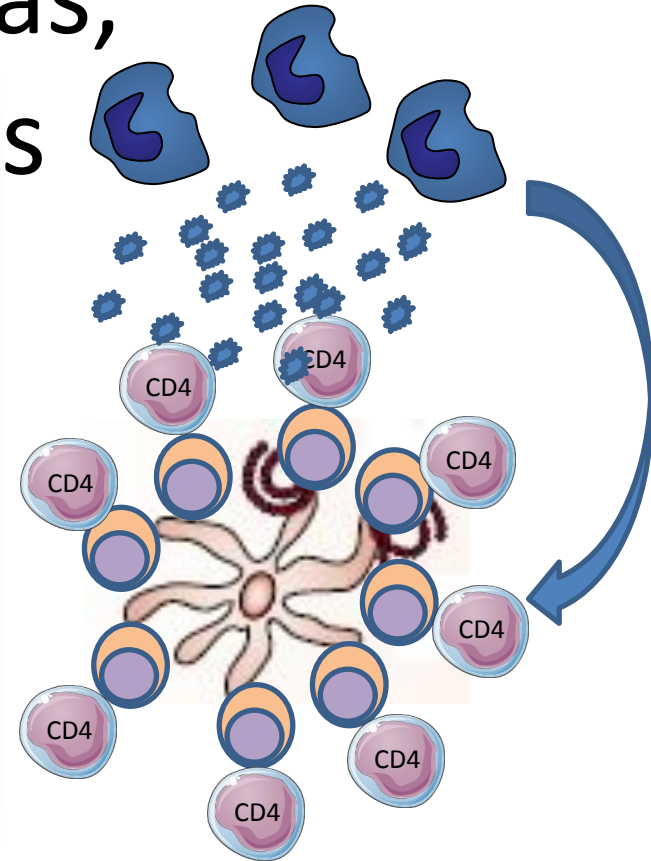
- IL-1 beta – piroptose
- Ativação BHE
- TNF- α - apoptose
- IFN- γ – produção NO
- Aumento MHC I e II
- IL-6
- IL-17 – Ativação da BHE
- IL-21
- IL-23
- GM-CSF
- NO
- MMPs
- Granzimas/Perforinas
- Anticorpos
- Neurotransmissores
- Glutamato

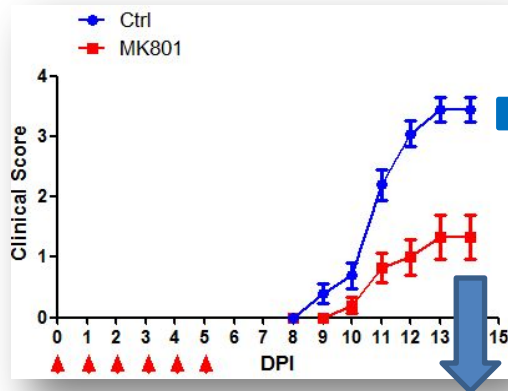


Progressão – Estabelecimento dos Folículos-like Produção de Citocinas,



Ps

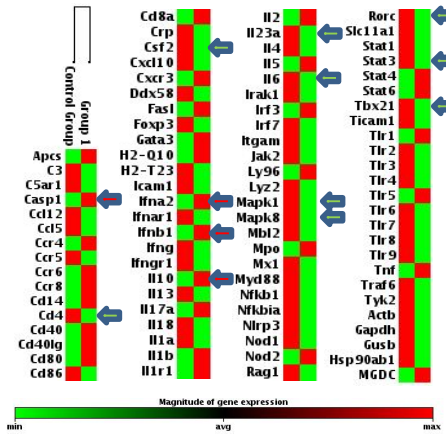




Controle



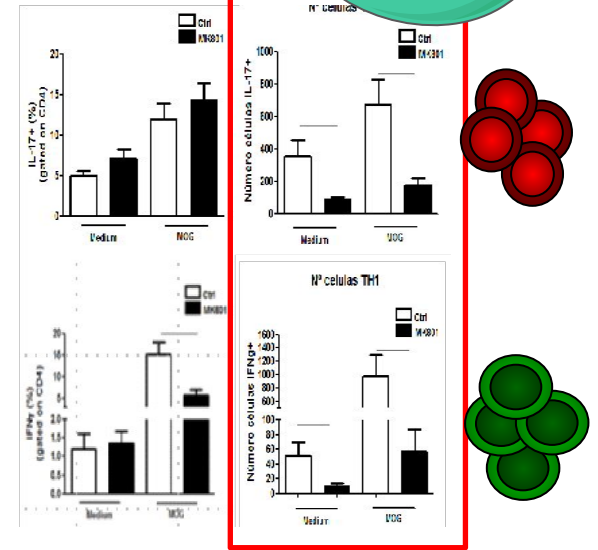
MK801



Glutamato

NMDAr

Th17
Th1



Abordagens Terapêuticas

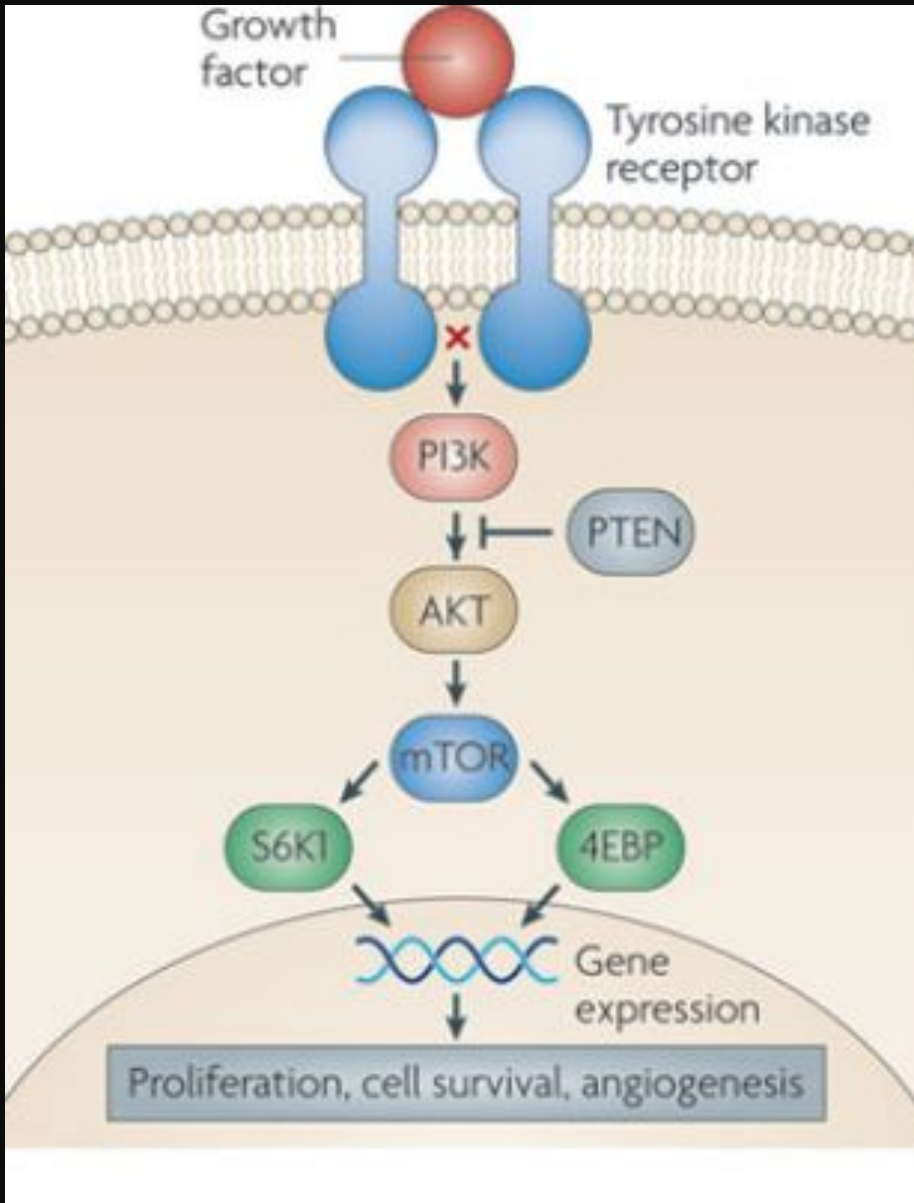
TABLE 18–5 Examples of Cytokine Antagonists in Clinical Use or Trials

Cytokine or Receptor Targeted	Predicted Biologic Effects Of Antagonist	Clinical Indications
TNF	Inhibits leukocyte migration into sites of inflammation	Rheumatoid arthritis, psoriasis, inflammatory bowel disease
IL-1	Inhibits leukocyte migration into sites of inflammation	Rare autoinflammatory syndromes, severe gout, rheumatoid arthritis
IL-6 and IL-6 receptor	Inhibits synthesis of acute-phase proteins, antibody responses?	Juvenile idiopathic arthritis, rheumatoid arthritis
IL-17	Inhibits leukocyte recruitment into sites of inflammation	Rheumatoid arthritis, psoriasis
p40 chain of IL-12 and IL-23	Inhibits T _H 1 and T _H 17 responses	Inflammatory bowel disease, psoriasis
IL-2 receptor (CD25)	Inhibits IL-2–mediated T cell proliferation	Acute graft rejection
IFN- α	May be multiple effects on T _H 1 differentiation, antibody production	Systemic lupus erythematosus
IL-4	Inhibits T _H 2 differentiation, IgE production	Asthma
IL-5	Inhibits eosinophil activation	Asthma

The table lists examples of antagonists against cytokines (antibodies or soluble receptors) that are approved for clinical use or in trials. IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

Pten deletion in RIP-Cre neurons protects against type 2 diabetes by activating the anti-inflammatory reflex

Linyuan Wang¹⁻³, Darren Opland⁴, Sue Tsai¹, Cynthia T Luk^{1,3,5}, Stephanie A Schroer¹, Margaret B Allison⁴, Andrew J Elia^{6,7}, Caren Furlonger⁷, Akira Suzuki⁸, Christopher J Paige^{2,7,9}, Tak W Mak^{2,6,7,9}, Daniel A Winer^{1,9-11}, Martin G Myers Jr⁴ & Minna Woo^{1-3,5,11}



Antagônica à IP3 Kinase

Pathogenesis

- Aumento tecido adiposo
- Extravazamento de lipídeos intra celulares
- Cristais de Colesterol – TLR-4
- Ceramida – NALP3
- Hiperfosforilação de receptores
- ISR – Insulin Receptor Substrate
Hiperfosforilação
- Músculo, hipotálamo, fígado

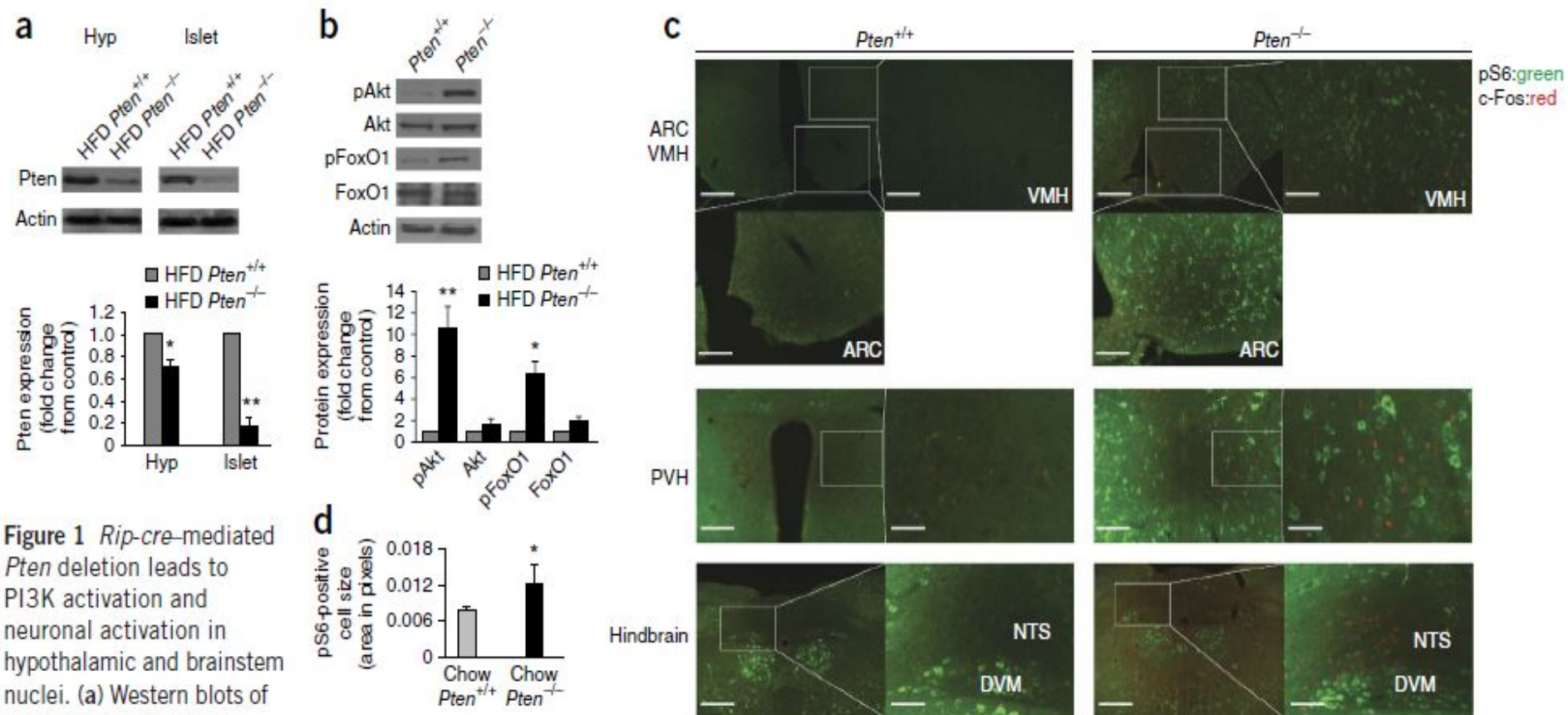


Figure 1 *Rip-cre*-mediated *Pten* deletion leads to PI3K activation and neuronal activation in hypothalamic and brainstem nuclei. (a) Western blots of *Pten* protein levels in

hypothalamic (Hyp) and islet lysates (top) and quantification (bottom) in *Rip-cre*⁺ *Pten*^{+/+} (*Pten*^{+/+}) and *Rip-cre*⁺ *Pten*^{flox/flox} (*Pten*^{-/-}) mice ($n = 6$ per group). (b) Western blots (top) and quantification (bottom) of whole hypothalamic pAkt, total Akt, pFoxO1 and total FoxO1 from *Pten*^{+/+} and *Pten*^{-/-} mice ($n = 6$ per group). (c) Representative immunofluorescent staining of pS6 (green) and c-Fos (red) in arcuate (ARC), ventromedial (VMH) and paraventricular (PVH) nuclei of the hypothalamus (20 \times magnification; scale bars, 100 μ m) and in NTS and DVM nuclei of the brainstem (10 \times magnification; scale bars, 200 μ m) from *Pten*^{+/+} and *Pten*^{-/-} mice. (d) Size of pS6-positive cells in pixels from the PVH nucleus ($n = 6$ per group). All data are presented as the means \pm s.e.m. * $P < 0.05$, ** $P < 0.01$ (independent-sample t test).

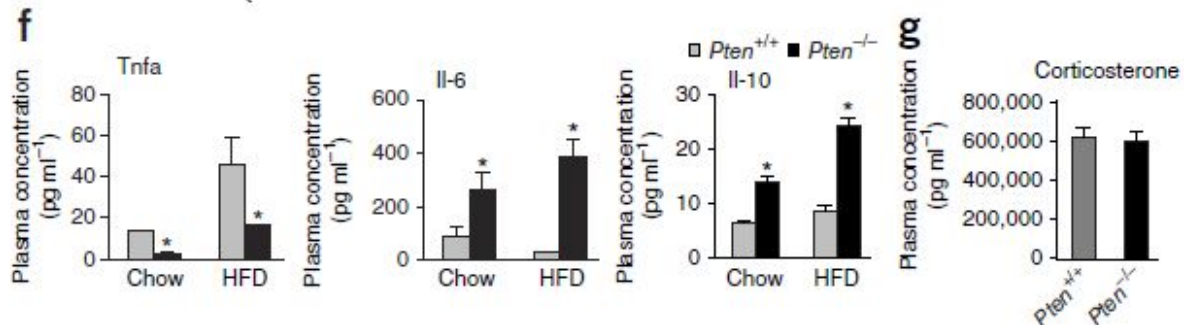
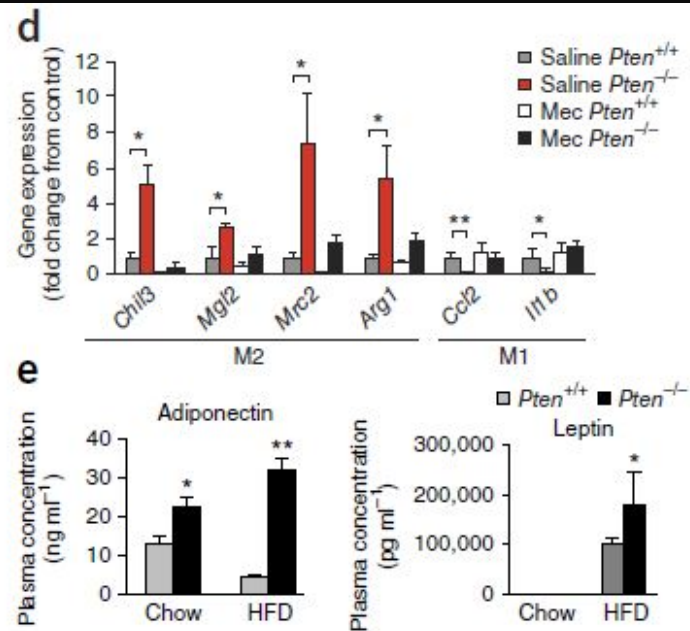
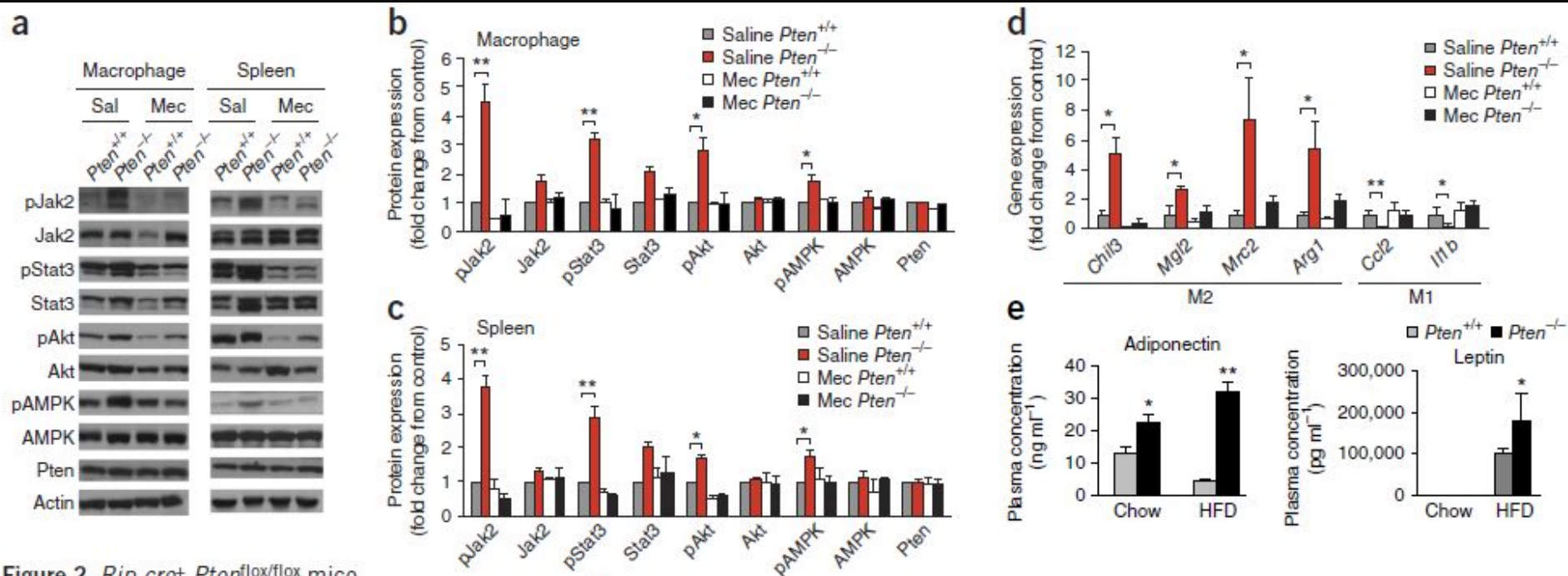


Figure 2 *Rip-cre*⁺ *Pten*^{flx/flx} mice exhibit predominant M2 macrophage polarization and decreased inflammation. (a) Western blots of peritoneal macrophages and splenic lysates for pJak2, total Jak2, pStat3, total Stat3, pAkt, total Akt, pAMPK, total AMPK and Pten levels in *Rip-cre*⁺ *Pten*^{+/+} (*Pten*^{+/+}) and *Rip-cre*⁺ *Pten*^{flx/flx} (*Pten*^{-/-}) mice on chow diet after treatment with either saline (Sal) or mecamlamine (Mec) ($n = 6$ per group).

(b,c) Quantification of protein levels in peritoneal macrophages (b) and splenic lysates (c) ($n = 6$ per group). (d) Quantitative PCR to indicate the transcript levels of genetic markers of M2 polarized macrophages (*Chil3*, *Mgl2*, *Mrc2* and *Arg1*) and M1 macrophages (*Ccl2* and *Il1b*) in *Pten*^{+/+} and *Pten*^{-/-} mice on chow diet after treatment with either saline or mecamlamine ($n = 6$ per group). (e-g) Fasting plasma adipokines adiponectin (left) and leptin (right) (e), cytokines Tnfa (left), Il-6 (center) and Il-10 (right) (f) and corticosterone (g) in *Pten*^{+/+} and *Pten*^{-/-} mice on chow or HFD ($n = 10$ per group). All data are presented as the means \pm s.e.m. * $P < 0.05$, ** $P < 0.01$ (independent-sample *t* test).

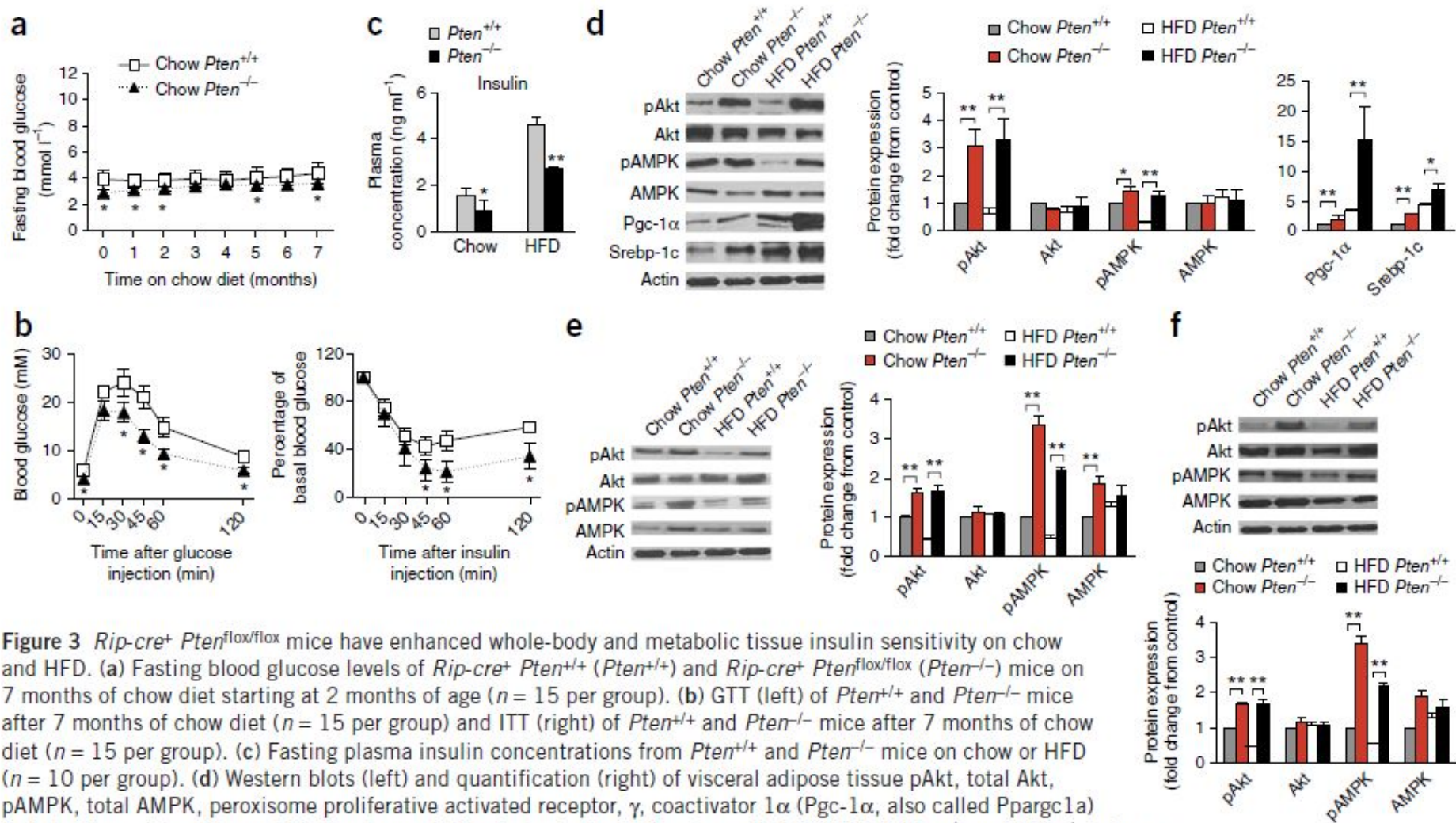


Figure 3 *Rip-cre*⁺ *Pten*^{fllox/fllox} mice have enhanced whole-body and metabolic tissue insulin sensitivity on chow and HFD. (a) Fasting blood glucose levels of *Rip-cre*⁺ *Pten*^{+/+} (*Pten*^{+/+}) and *Rip-cre*⁺ *Pten*^{fllox/fllox} (*Pten*^{-/-}) mice on 7 months of chow diet starting at 2 months of age (*n* = 15 per group). (b) GTT (left) of *Pten*^{+/+} and *Pten*^{-/-} mice after 7 months of chow diet (*n* = 15 per group) and ITT (right) of *Pten*^{+/+} and *Pten*^{-/-} mice after 7 months of chow diet (*n* = 15 per group). (c) Fasting plasma insulin concentrations from *Pten*^{+/+} and *Pten*^{-/-} mice on chow or HFD (*n* = 10 per group). (d) Western blots (left) and quantification (right) of visceral adipose tissue pAkt, total Akt, pAMPK, total AMPK, peroxisome proliferative activated receptor, γ , coactivator 1 α (Pgc-1 α , also called Ppargc1a) and sterol regulatory element binding transcription factor 1 (Srebp-1c, also called Srebf1) of *Pten*^{+/+} and *Pten*^{-/-} mice on chow or HFD (*n* = 6 per group). (e,f) Western blots (left) and quantification (right) of pAkt, total Akt, pAMPK and total AMPK in liver (e) and muscle (f; top and bottom, respectively) of *Pten*^{+/+} and *Pten*^{-/-} mice on chow or HFD (*n* = 6 per group). All data are presented as the means \pm s.e.m. **P* < 0.05, ***P* < 0.01 (independent-sample *t* test).

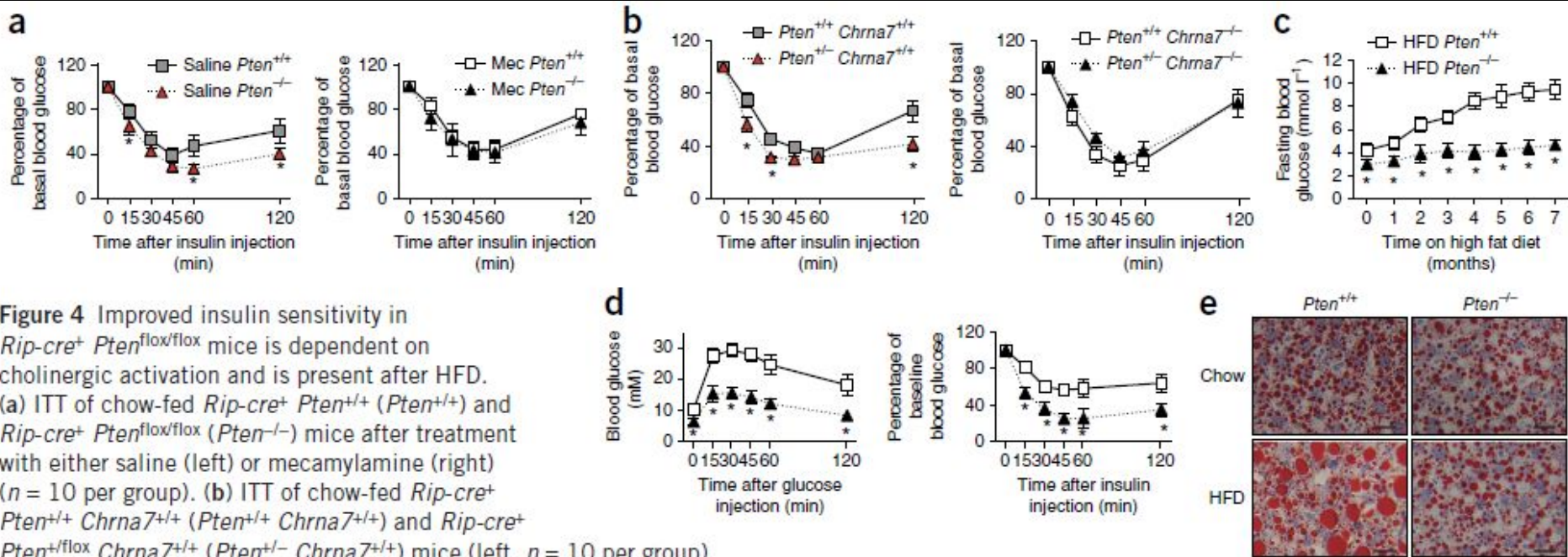


Figure 4 Improved insulin sensitivity in

Rip-cre⁺ Pten^{flox/flox} mice is dependent on cholinergic activation and is present after HFD.

(a) ITT of chow-fed *Rip-cre⁺ Pten^{+/+}* ($Pten^{+/+}$) and *Rip-cre⁺ Pten^{flox/flox}* ($Pten^{-/-}$) mice after treatment with either saline (left) or mecamylamine (right) ($n = 10$ per group). (b) ITT of chow-fed *Rip-cre⁺ Pten^{+/+} Chrna7^{+/+}* ($Pten^{+/+} Chrna7^{+/+}$) and *Rip-cre⁺ Pten^{flox/flox} Chrna7^{+/+}* ($Pten^{-/-} Chrna7^{+/+}$) mice (left, $n = 10$ per group).

ITT of chow-fed *Rip-cre⁺ Pten^{+/+} Chrna7^{-/-}* ($Pten^{+/+} Chrna7^{-/-}$) and

Rip-cre⁺ Pten^{flox/flox} Chrna7^{-/-} ($Pten^{-/-} Chrna7^{-/-}$) mice (right, $n = 7$ per group). (c) Fasting blood glucose levels of $Pten^{+/+}$ and $Pten^{-/-}$ mice on 7 months of HFD starting at 2 months of age ($n = 15$ per group).

(d) GTT of $Pten^{+/+}$ and $Pten^{-/-}$ mice after 7 months of HFD (left, $n = 15$ per group) and ITT of $Pten^{+/+}$ and $Pten^{-/-}$ mice after 7 months of HFD (right, $n = 15$ per group).

(e) Representative Oil Red O-stained liver sections of $Pten^{+/+}$ and $Pten^{-/-}$ mice on either chow or HFD (scale bars, 80 μ m).

All data are presented as the means \pm s.e.m. * $P < 0.05$, ** $P < 0.01$ (independent-sample t test).

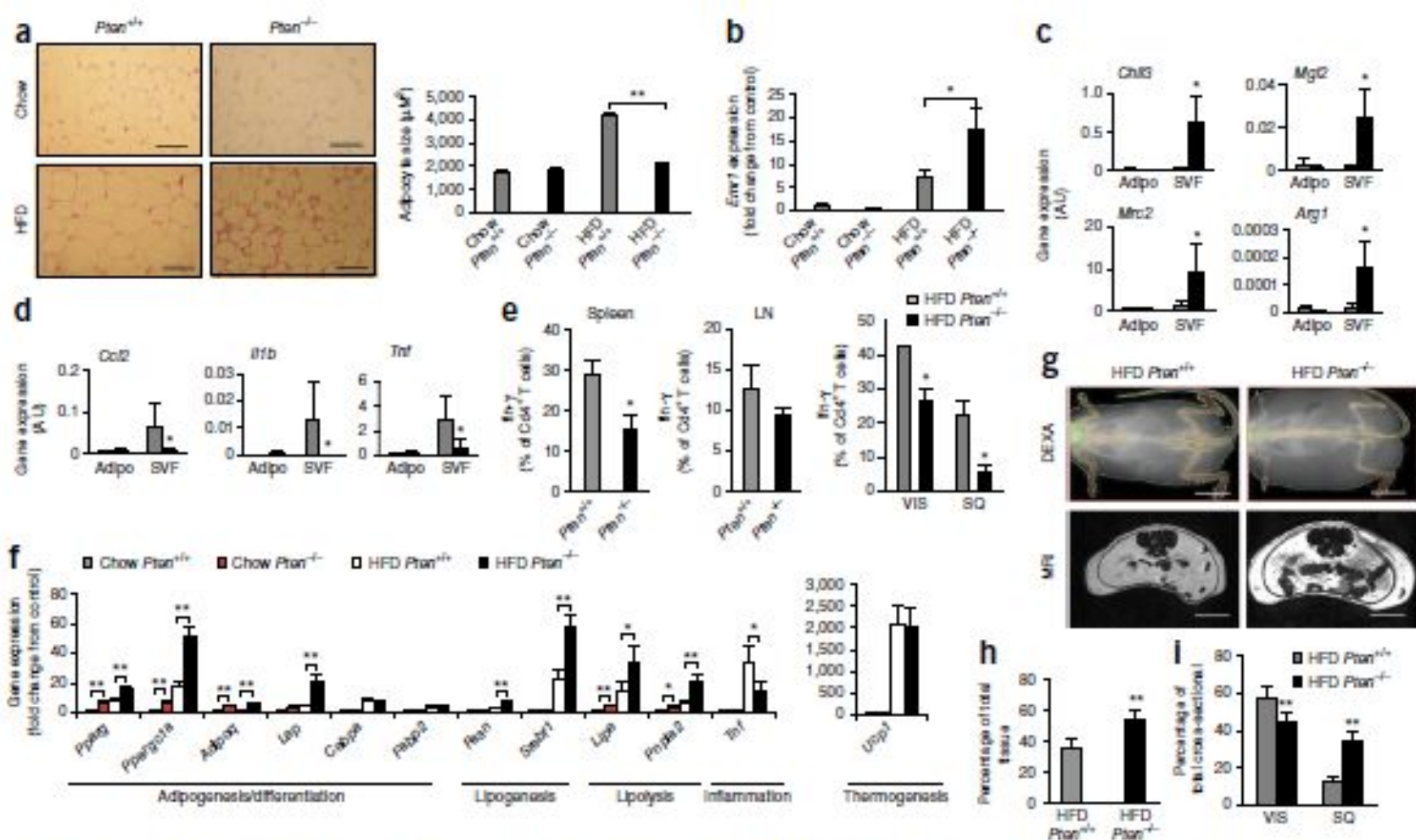


Figure 5 HFD-fed *Rip-cre*⁺ *Pten*^{flox/flox} mice demonstrate greater M2 macrophage infiltration and adipogenesis in the visceral compartment. (a) Representative H&E-stained visceral adipose tissue sections (left; scale bars, 200 μm) and quantification of adipocyte size (right) in *Rip-cre*⁺ *Pten*^{+/+} (*Pten*^{+/+}) and *Rip-cre*⁺ *Pten*^{flox/flox} (*Pten*^{-/-}) mice on chow or HFD (*n* = 6 per group). (b) Quantitative PCR of *Emr1* transcript levels in visceral adipocytes of *Pten*^{+/+} and *Pten*^{-/-} mice on chow or HFD (*n* = 6 per group). (c) Quantitative PCR of *Chi3*, *Mgl2*, *Mrc2* and *Arg1* transcript levels of adipocytes (Adipo) and the SVF from HFD-fed *Pten*^{+/+} and *Pten*^{-/-} mice (*n* = 6 per group). AU, arbitrary units. (d) Quantitative PCR of *Ccl2*, *Ilf1b* and *Tnf* transcript levels of adipocytes and the SVF from HFD-fed *Pten*^{+/+} and *Pten*^{-/-} mice (*n* = 6 per group). (e) Phorbol myristate acetate (PMA)-stimulated Ifn-γ production by CD4⁺ T cells isolated from the spleen (left), lymph node (LN) (center) and visceral (VIS) and subcutaneous (SQ) adipose tissue (right) of two to three pooled *Pten*^{+/+} and *Pten*^{-/-} mice on HFD (*n* = 4 per group). (f) Gene expression of markers for adipogenesis (*Pparg*, *Ppargc1a*, *Adipoq*, *Lep*, *Cebpa* and *Fabp4*), lipogenesis (*Fasn* and *Srebf1*), lipolysis (*Lipe* and *Pnpla2*), inflammation (*Tnf*) and thermogenesis (*Ucp1*) (*n* = 6 per group). (g) Representative magnetic resonance imaging (MRI) and dual-energy X-ray absorptiometry (DEXA) images of *Pten*^{+/+} and *Pten*^{-/-} mice after 7 months on HFD starting at 2 months of age (scale bars, 75 mm). (h) Percentage body fat of *Pten*^{+/+} and *Pten*^{-/-} mice after 7 months of HFD by DEXA (*n* = 5). (i) Quantification of VIS and SQ adipose tissue in cross-sectional areas of MRIs of *Pten*^{+/+} and *Pten*^{-/-} mice (*n* = 5). All data are presented as the means ± s.e.m. **P* < 0.05, ***P* < 0.01 (independent-sample *t* test).

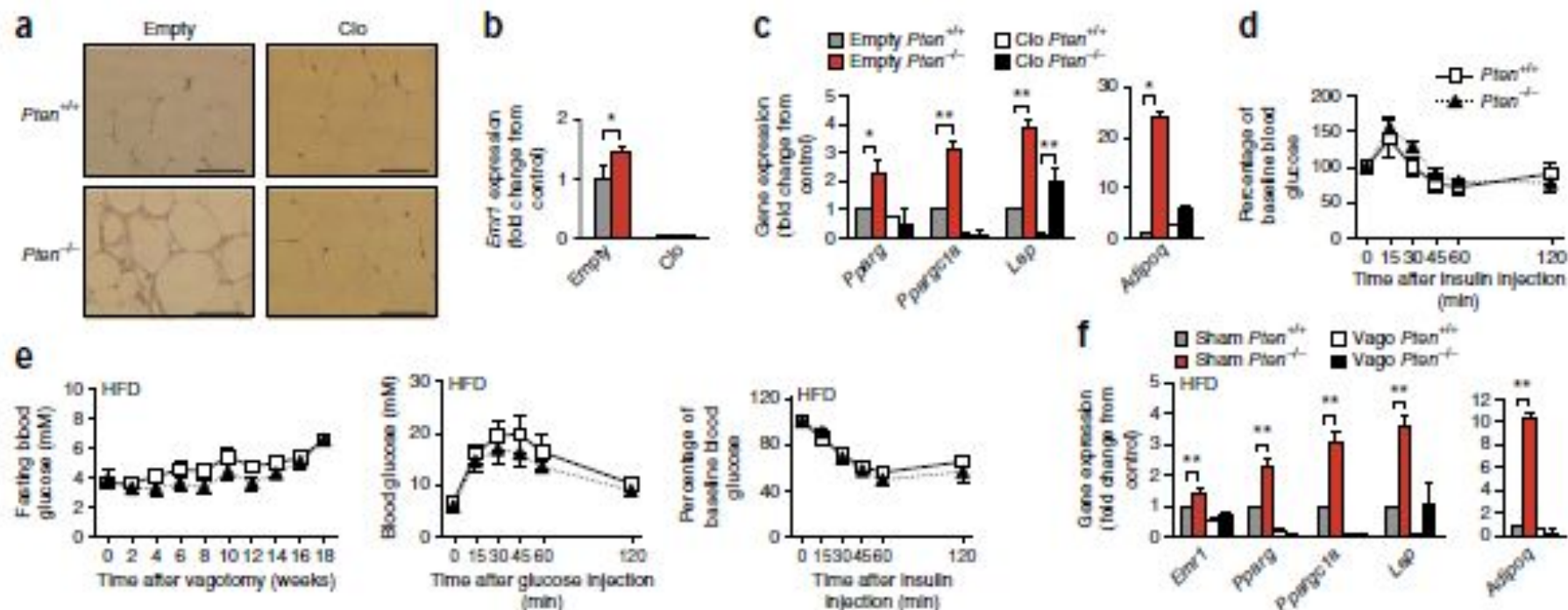


Figure 6 Macrophage depletion or vagotomy attenuates visceral adipogenesis and peripheral insulin sensitivity.

(a) Representative immunohistochemical staining of F4/80 in visceral adipose tissue from chow-fed *Rip-cre⁺ Pten^{+/+}* (*Pten^{+/+}*) and *Rip-cre⁺ Pten^{lox/lox}* (*Pten^{-/-}*) mice after injection of either empty liposome (Empty) or liposome-encapsulated clodronate (Clo) (scale bars, 200 μ m). (b) Quantitative PCR of *Emr1* (F4/80) transcript levels in visceral adipose tissue of chow-fed *Pten^{+/+}* and *Pten^{-/-}* mice after injection of either empty liposome or clodronate ($n = 6$ per group). (c) ITT of chow-fed *Pten^{+/+}* and *Pten^{-/-}* mice after clodronate injection ($n = 10$ per group). (d) Quantitative PCR of adipogenic marker (*Pparg*, *Pparg1a*, *Lep* and *Adipoq*) gene expression levels in visceral adipose tissue of chow-fed *Pten^{+/+}* and *Pten^{-/-}* mice after injection of either empty liposome or clodronate ($n = 3$ per group). (e) Fasting blood glucose levels (left), GTT (center) and ITT (right) of vagotomized *Pten^{+/+}* and *Pten^{-/-}* mice after 5 months of HFD ($n = 7$). (f) Quantitative PCR of *Emr1*, *Pparg*, *Pparg1a*, *Lep* and *Adipoq* gene expression levels in visceral adipose tissue of vagotomized (Vago) *Pten^{+/+}* and *Pten^{-/-}* mice on HFD ($n = 6$ per group). (g) Quantitative PCR of *Mgl2*, *Mrc2*, *Ccl2* and *Irf1b* gene expression levels in visceral adipose tissue of vagotomized *Pten^{+/+}* and *Pten^{-/-}* mice on HFD normalized to *Emr1* levels ($n = 6$ per group). (h) Schematic representation of the mechanism of PI3K activation in RIP-Cre neurons mediating protection against type 2 diabetes (T2DM). All data are presented as the means \pm s.e.m. * $P < 0.05$, ** $P < 0.01$ (independent-sample t test).

