



**86e JOURNÉE FRANÇAISE DE MÉDECINE  
PATHOLOGIES MUSCULAIRES EN MÉDECINE INTERNE  
22 Mai 2015**

# **Myasthénie gravis : physiopathologie et nouveaux auto-anticorps**

**Rozen Le Panse**

Myologie Centre de Recherche



# Characteristics of autoimmune MG

- **Autoimmune myasthenia** ≠ congenital myasthenia
- **Rare disease** (prevalence: 1/20.000; incidence: 2-5 cases/year/million)
- **Neuromuscular** disease: defective transmission between nerve and muscle
- **Fatigable muscle weakness** of skeletal muscles: ocular - respiratory muscles
- **Auto-antibodies** against muscle components of the neuromuscular junction

# Classification of MG patients

**Affected  
muscles**



Ocular MG



Generalised MG

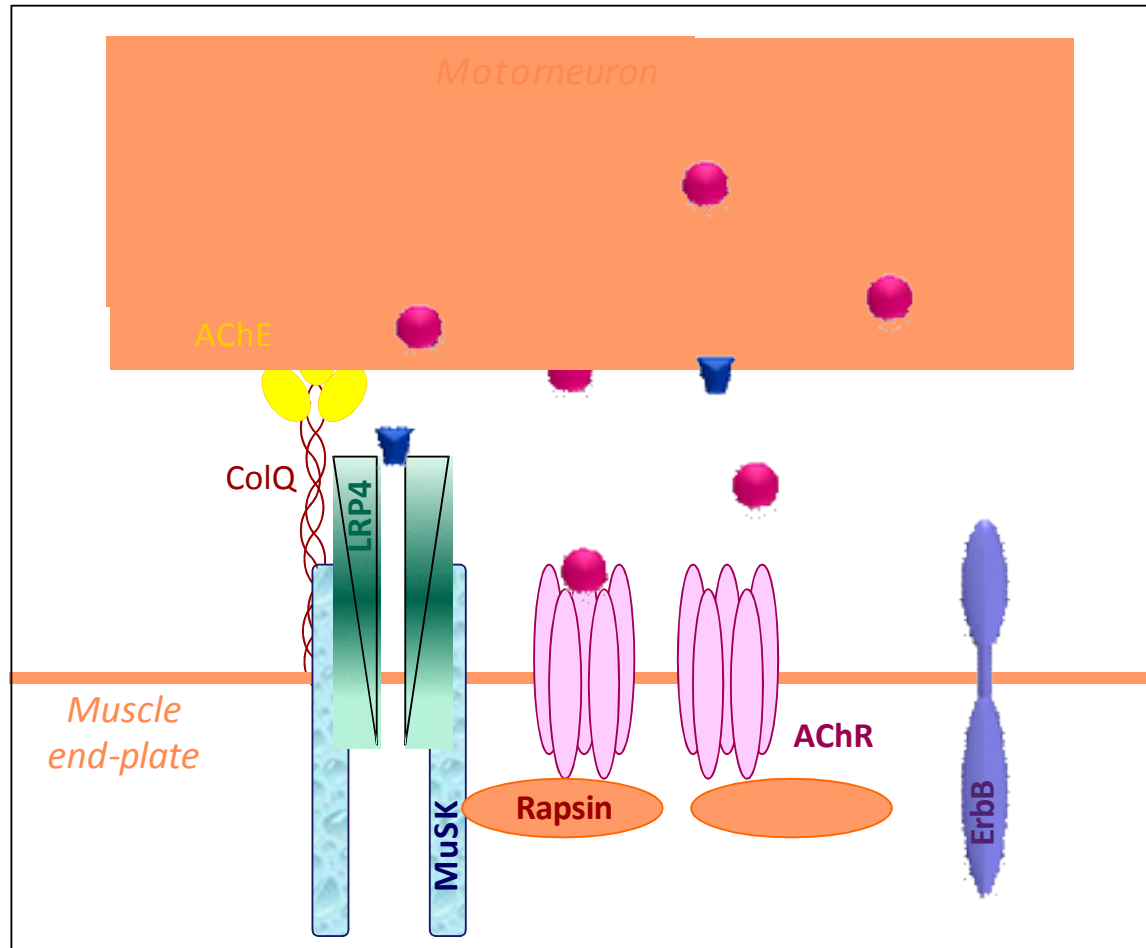
**Age of onset  
< 40-45 >**

Early onset  
Late onset

**Specificity of  
autoantibodies**

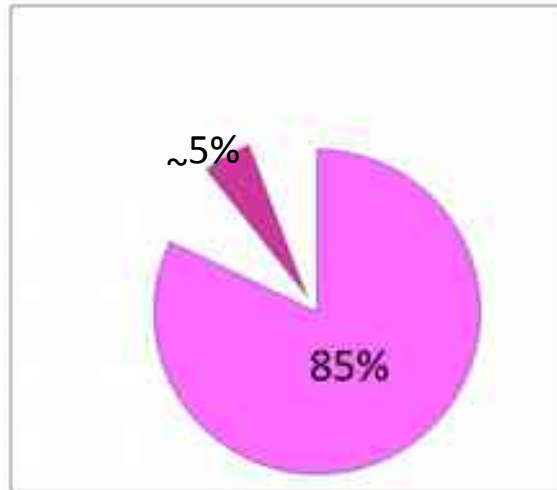
**Thymic  
abnormalities**

# Anti-AChR antibodies

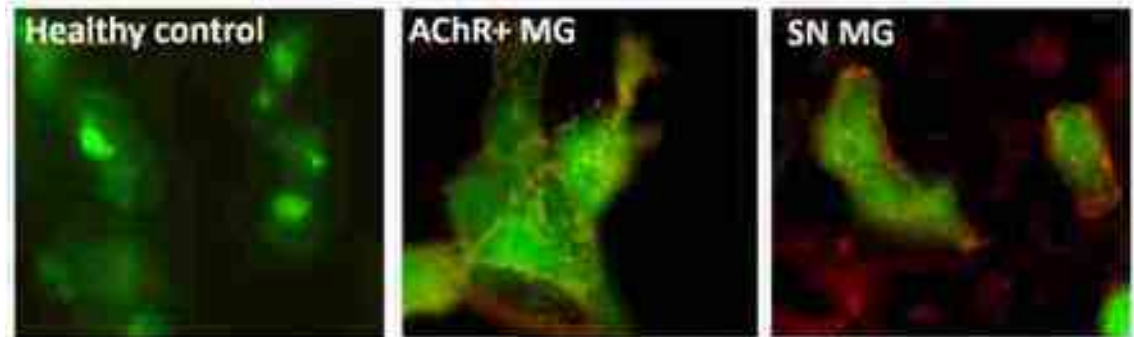


Simplified scheme of the neuromuscular junction

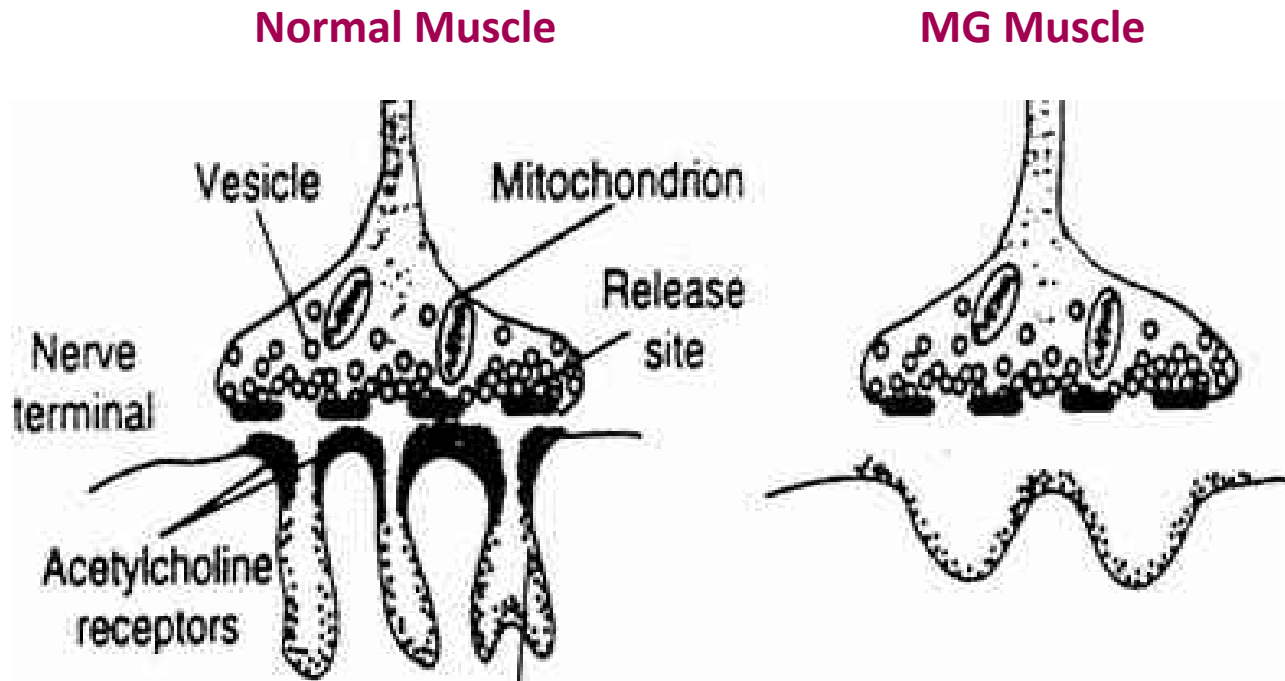
# Anti-AChR antibodies



- AChR (*Appel et al, 1976*)
  - > classical immunoprecipitation assay
- Clustered-AChR (*Leite et al, 2008*)
  - > cell-based assay on clustered-AChR



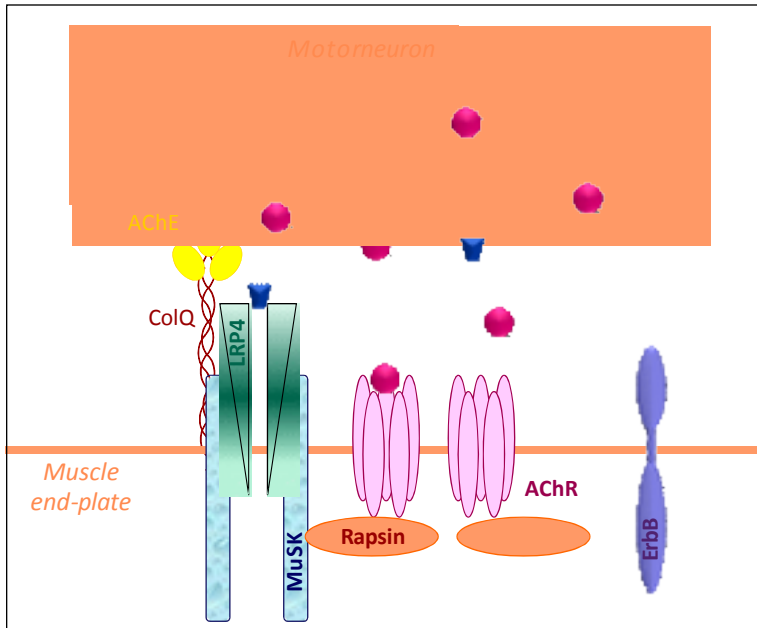
# What happens in the muscle of MG patients?



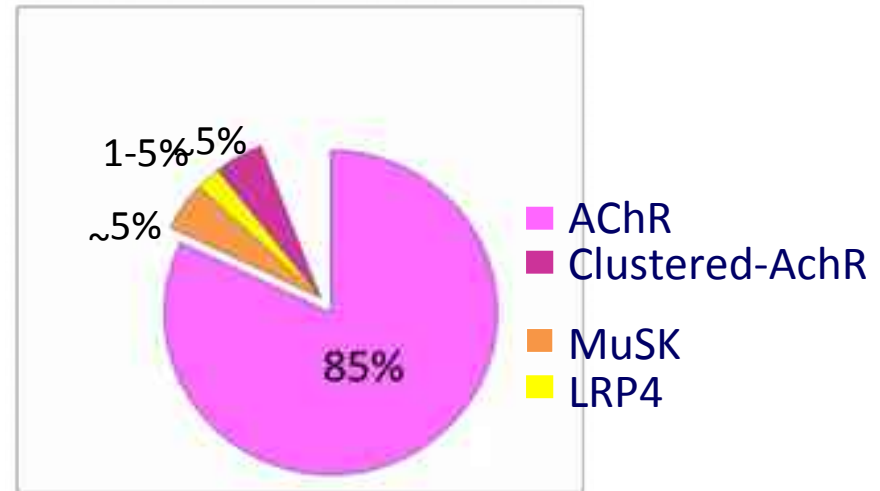
## Mechanisms of action of anti-AChR antibodies

- Accelerated degradation of AChR (*Drachman et al. 1977*)
- Complement mediated damage (*Engel et al. 1976*)
- Blocking antibodies (*Howard et al. 1987*)

# Anti-MuSK or anti-LRP4 antibodies

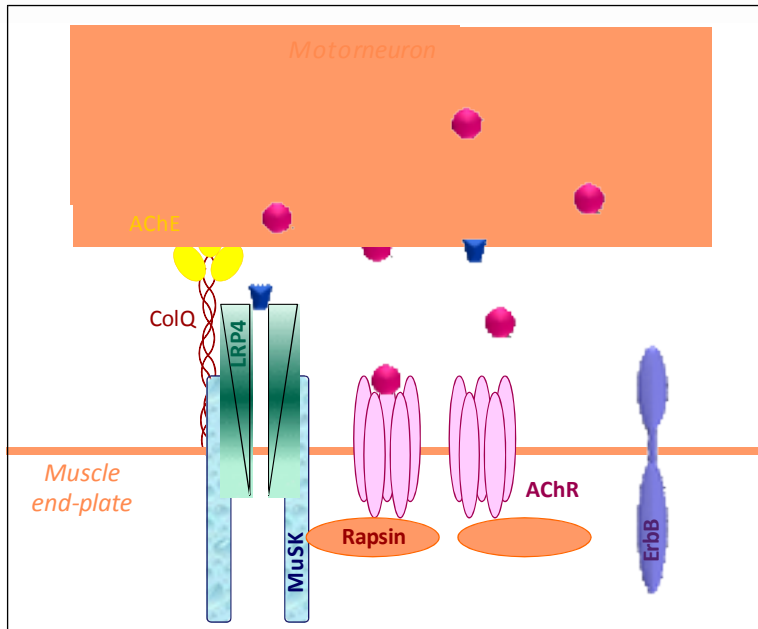


Simplified scheme of the neuromuscular junction

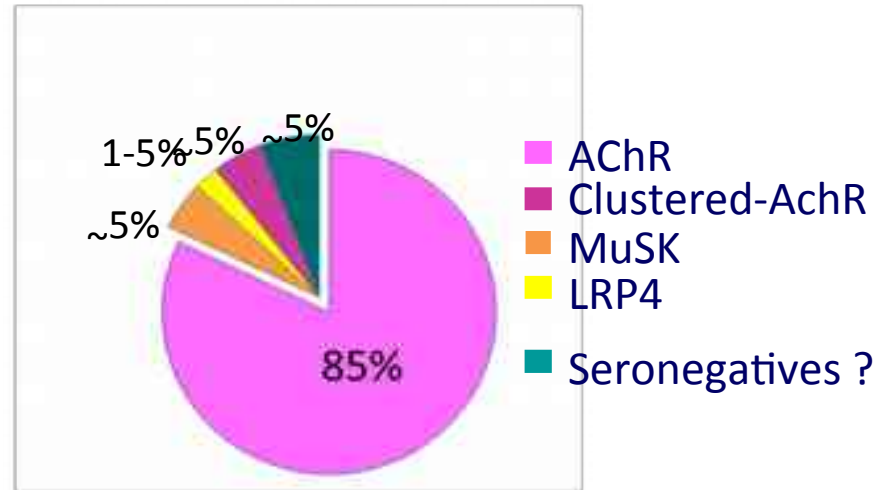


- MuSK for Muscle Specific Kinase
- LRP4 for Low-density lipoprotein Receptor-related Protein 4
- Agrin interacts with the LRP4-MuSK complex
- Agrin, MuSK and LRP4 are necessary for NMJ formation and the AChR clusterization

# Anti-MuSK antibodies



Simplified scheme of the neuromuscular junction



- Agrin (Gasperi et al, 2014; Zang et al, 2014)

Always detected in combination with anti-MuSK, LRP4, or AChR antibodies.

- Cortactin (Gallardo et al, 2014)

A protein acting downstream from agrin/MuSK promoting AChR clustering



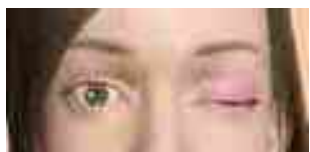
# Characteristics of autoantibodies

Antibodies to	AChR	AChR low affinity	MuSK	LRP4
Discovered by	Appel et al (1976)	Leite et al (2008)	Hoch et al (2001)	Higuchi et al (2011)
Frequency of patients	85%	3%	4%	2%
Pathogenicity	Animal model and in vitro	In vitro	Animal model and in vitro	Animal model and in vitro
Isotypes	IgG1, IgG3	IgG1	IgG4	IgG1-IgG3
Role of complement	Yes	Probable	No	Yes
Correlation with severity	No	?	Yes	?

**Double seropositive patients (AChR-MuSK, AChR-LRP4 or MuSK-LRP4)**

# Classification of MG patients

**Affected  
muscles**



Ocular MG



Generalised MG

**Age of onset  
< 40-45 >**

Early onset  
Late onset

**Specificity of  
autoantibodies**

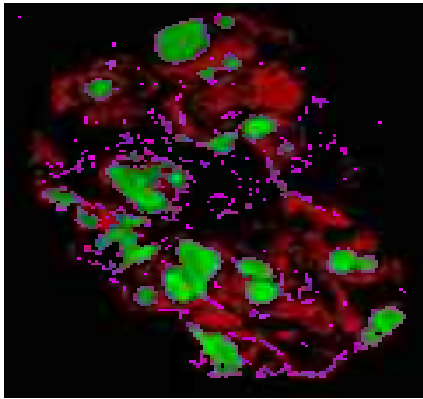
AChR  
MuSK  
LRP4

**Thymic  
abnormalities**

AChR  
*LRP4 ?*

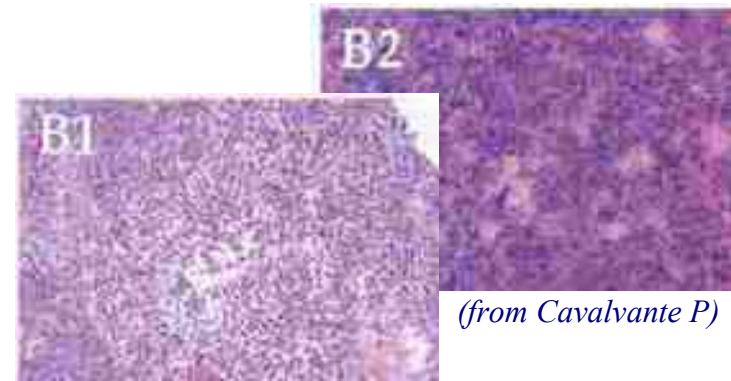
# Implication of the thymus in Myasthenia Gravis (MG)

## Follicular hyperplasia

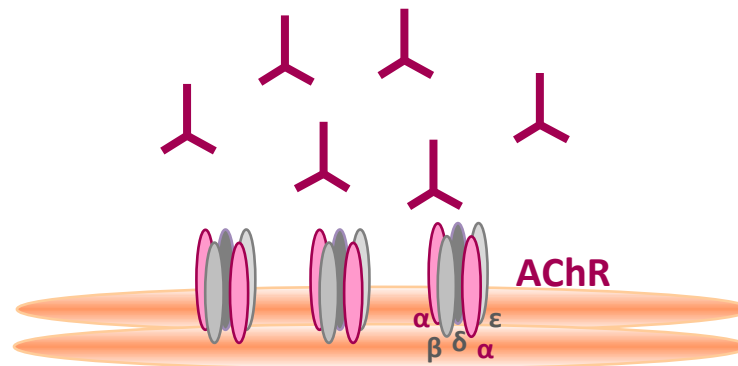


Early-onset < 40  
Mostly women

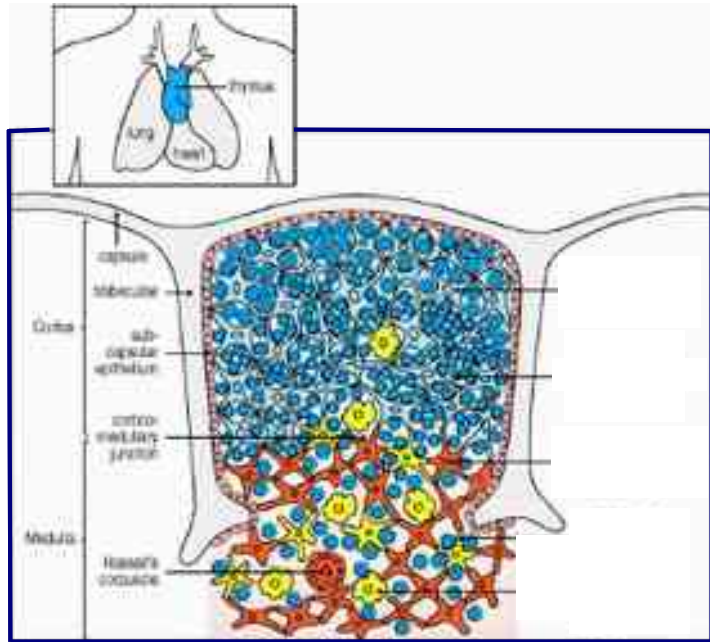
## Thymoma



Late-onset >50  
Men and women



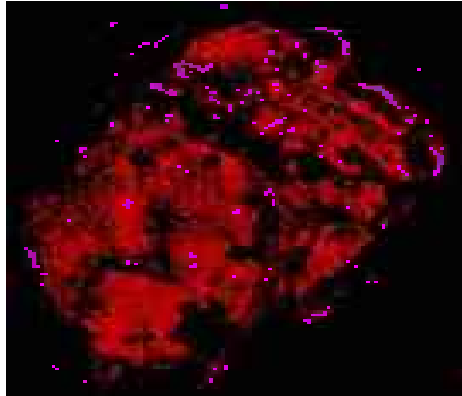
# The normal thymus



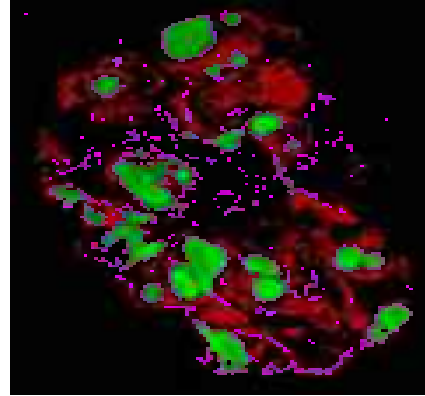
- Maturation and education of T cells
- Central role of thymic epithelial cells (TECs)
- mTECs expressed tissue-specific antigens (TSAs)
- mTECs expressed AChR subunits

# Thymic hyperplasia in AChR<sup>+</sup> EOMG

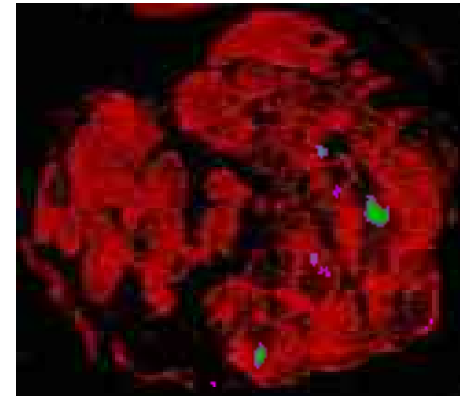
Adult control



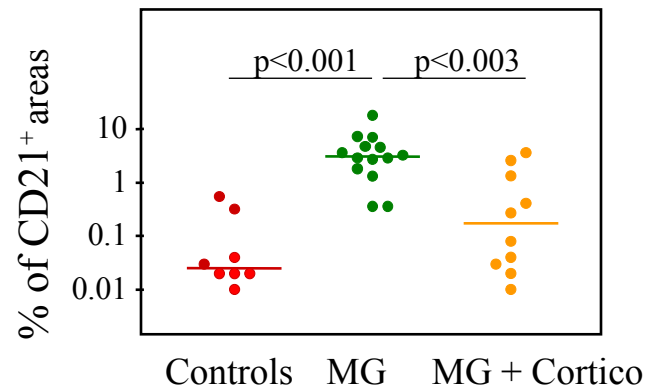
MG



MG + corticoids

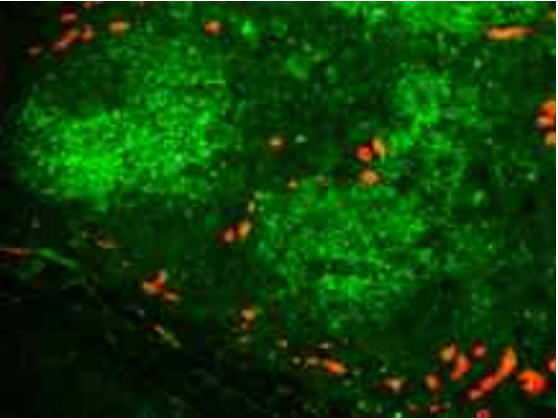
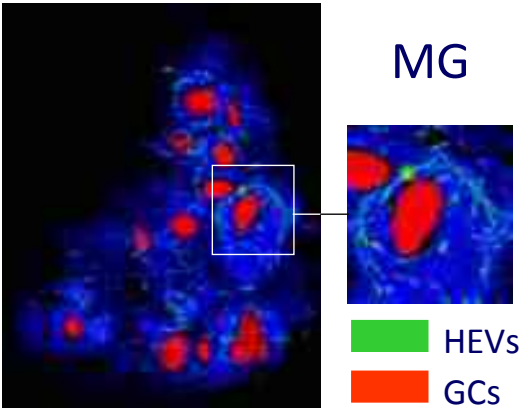


- Epithelial cells (keratin labeling)
- Germinal centers (CD21 labeling)



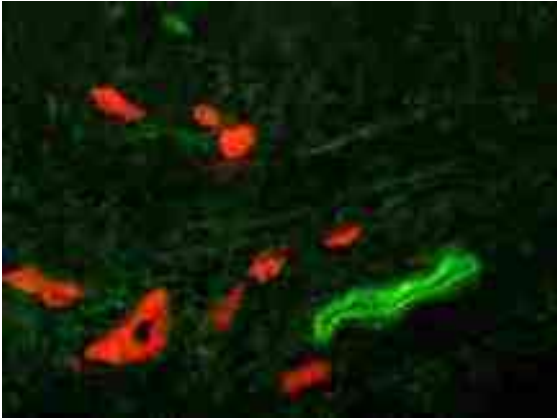
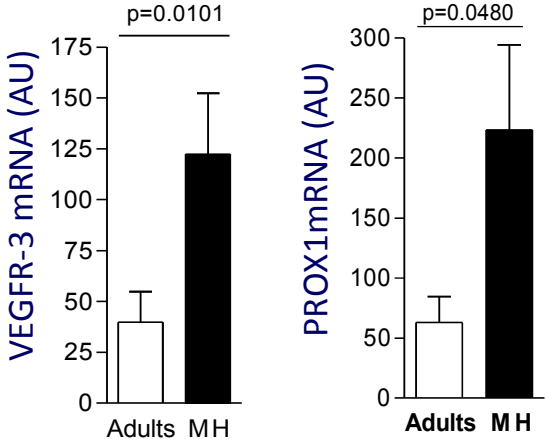
# Active angiogenic processes in AChR+ EOMG

## Ectopic HEV development



HEVs GCs

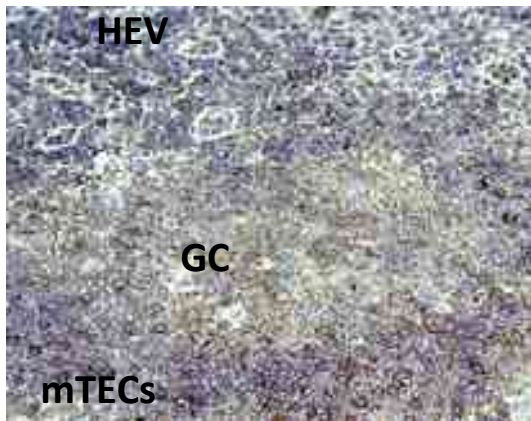
## Lymphangiogenesis



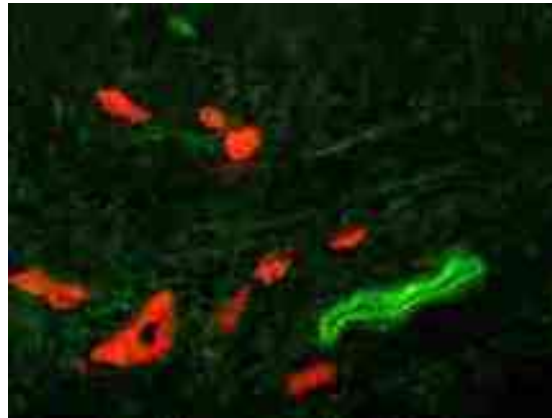
HEVs LV

# Increased expression of chemokines in AChR<sup>+</sup> EOMG

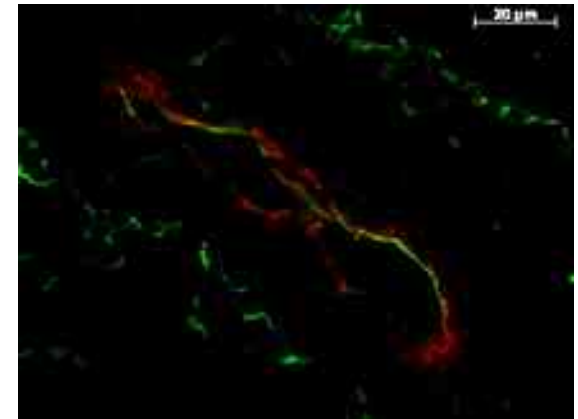
**CXCL13**  
by  
thymic epithelial cells



**CCL21**  
by  
lymphatic vessels



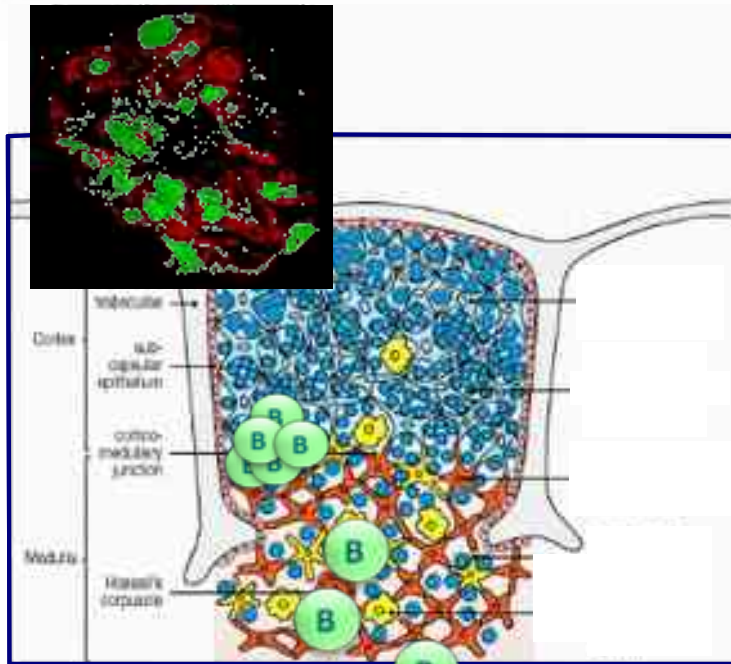
**SDF1/CXCL12**  
by  
HEVs



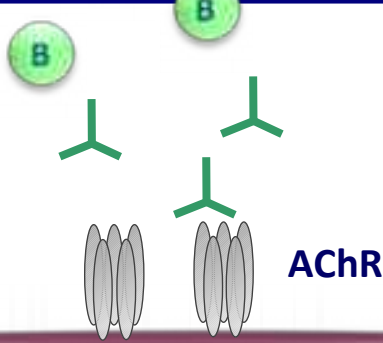
**Intense cellular trafficking in and out of the MG thymus**

*Méraouna et al. Blood 2006*  
*Berrih-Aknin et al. Ann. Neurol. 2008*  
*Weiss et al. Immunobiol. 2012*

# The pathological thymus in EOMG



- Germinal center development
- Anti-AChR autoreactive T cells
- B cells producing anti-AChR antibodies
- Active angiogenic processes
- Chemokine involvement



**Why myasthenia gravis ?**



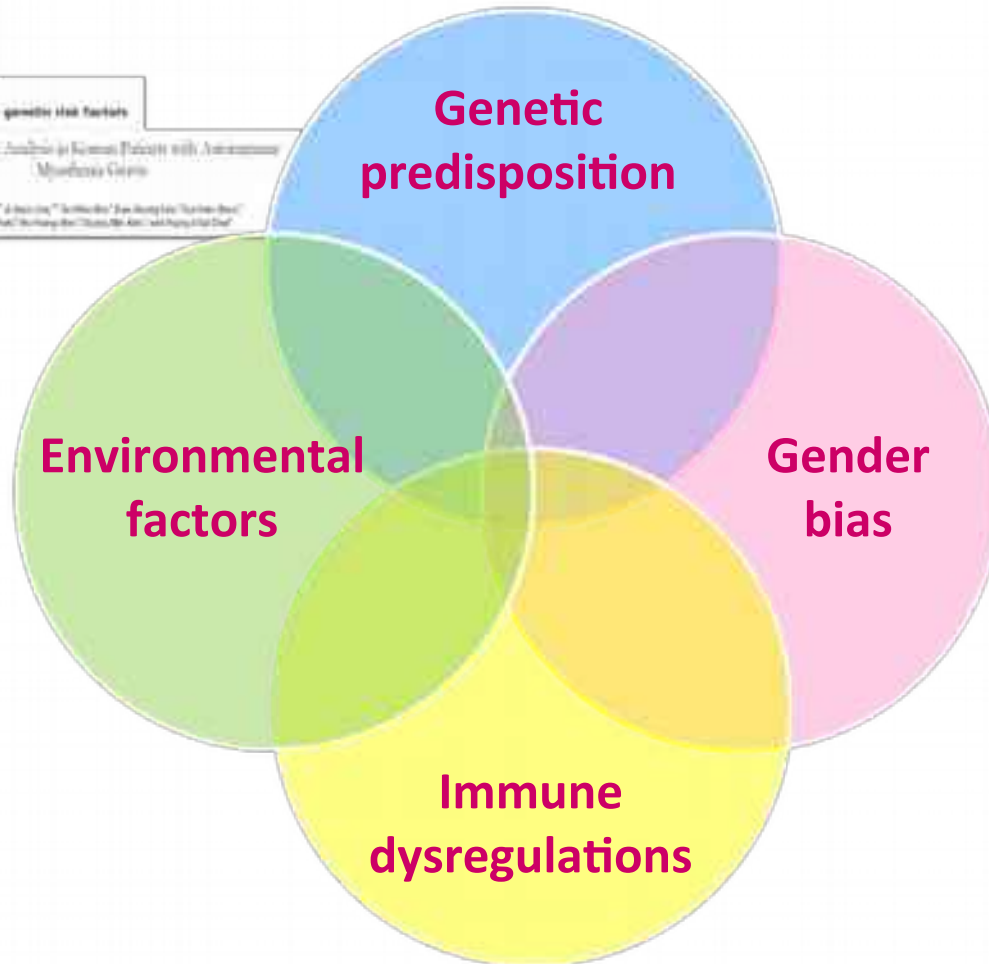
# Multifactorial disease

## Polymorphisms on HLA and other genes

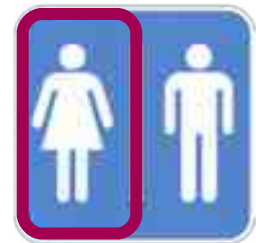
Risk for Myasthenia Gravis Maps to a <sup>157</sup>Pro→Ala Change in TNIP1 and to Human Leukocyte Antigen-B\*08

ESY1 and SAP1, via NLR pathway, are genetic risk factors for myasthenia gravis

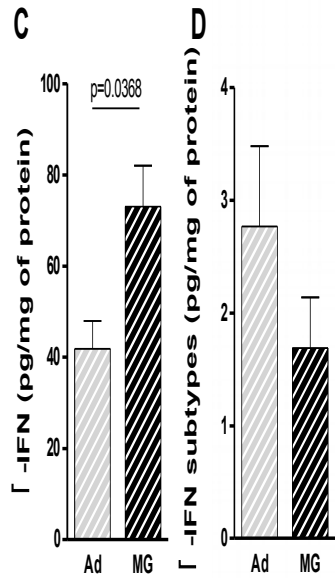
Whole Genome Analysis in German Patients with Antinuclear Myasthenia Gravis



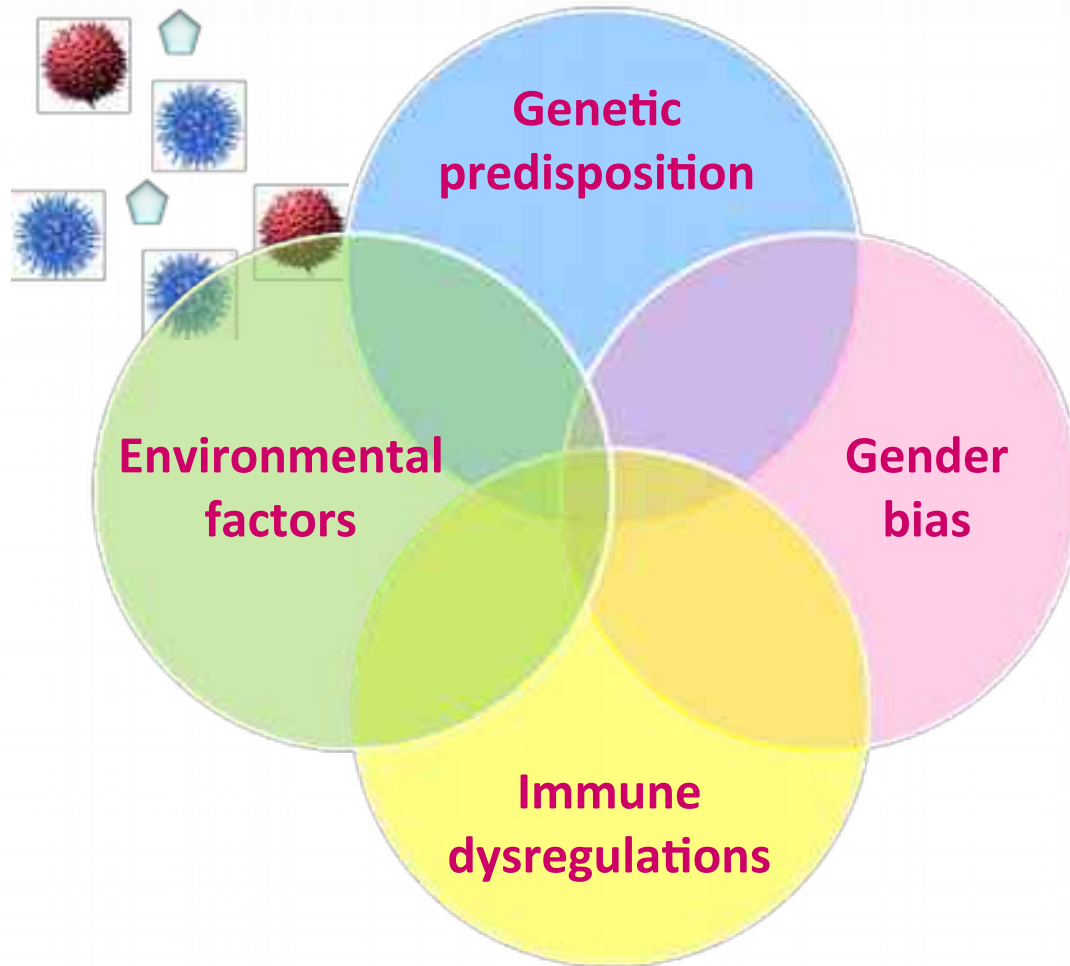
Female predisposition  
in EOMG



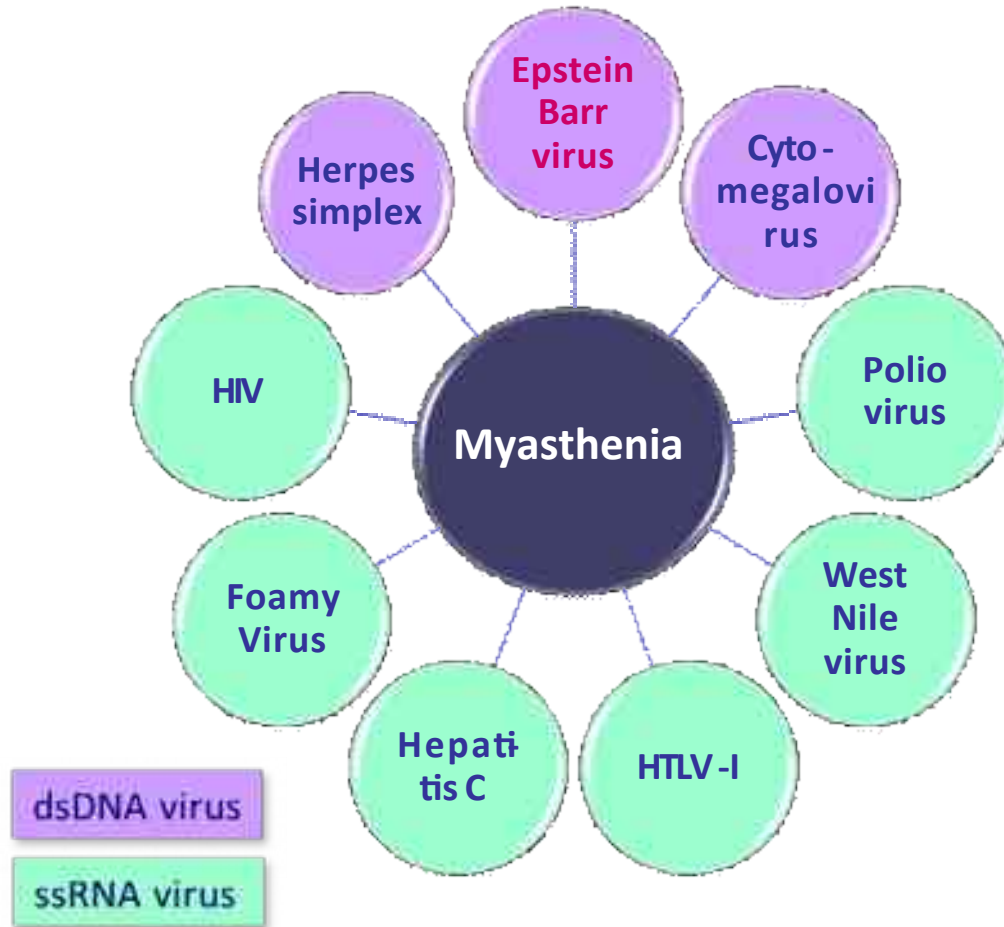
# Multifactorial disease



**Search for  
a viral signature**

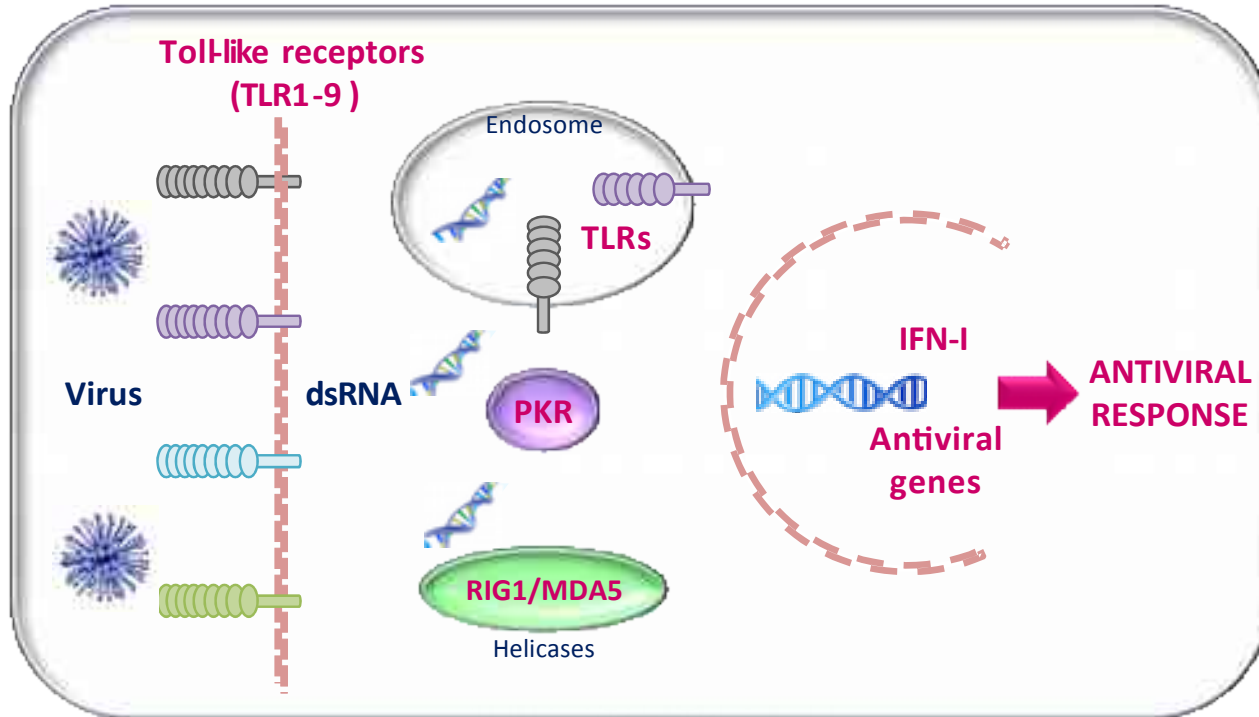


# Myasthenia and viral infections



# Toll-Like Receptors

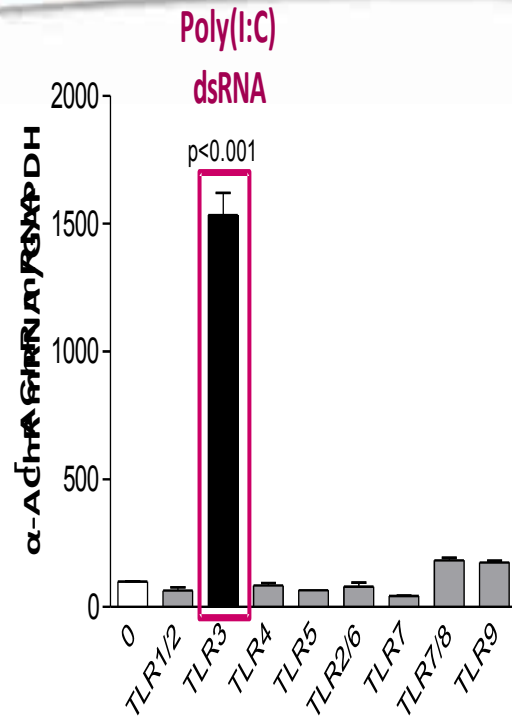
TLRs = Receptors for pathogen-associated molecular patterns



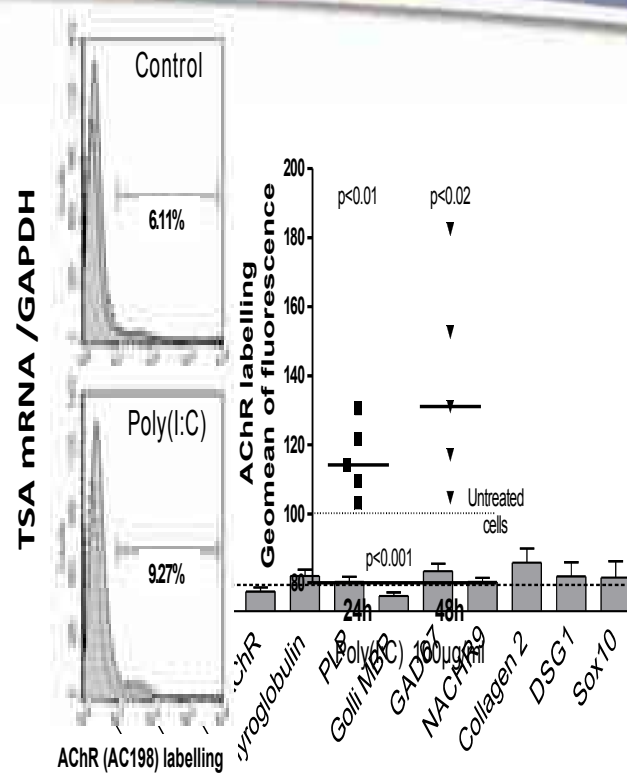
Effects of TLR agonists on thymic epithelial cells ?



# TLR agonist effects on TSA expression



TECs treated with TLR agonists



TECs treated with Poly(I:C) 100  $\mu$ g/ml

**Poly(I:C) selectively induced  $\alpha$ -AChR**

# Effects of Poly(I:C) injections in mice



Poly(I:C) (*ip*)

After 1 week  
Thymic changes

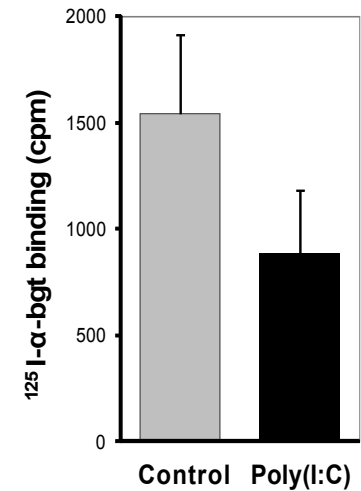
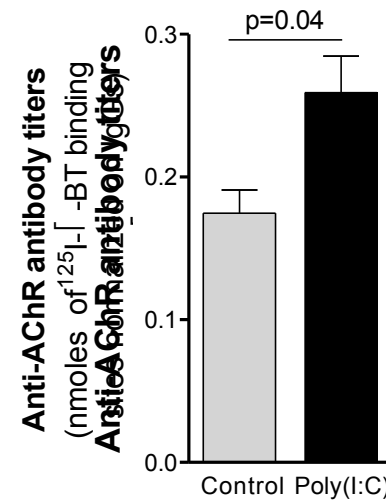
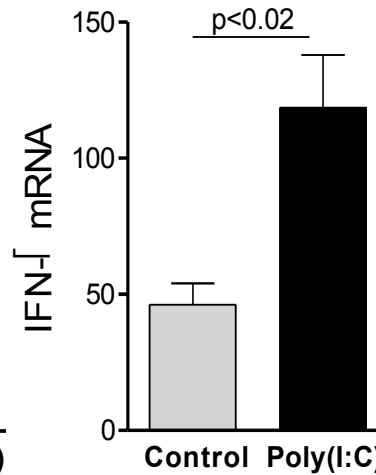
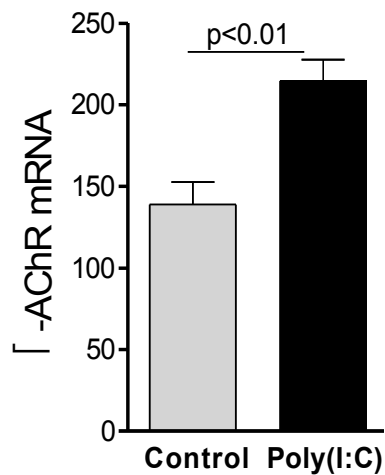
After 6 weeks  
Peripheral changes

$\alpha$ -AChR

IFN- $\beta$

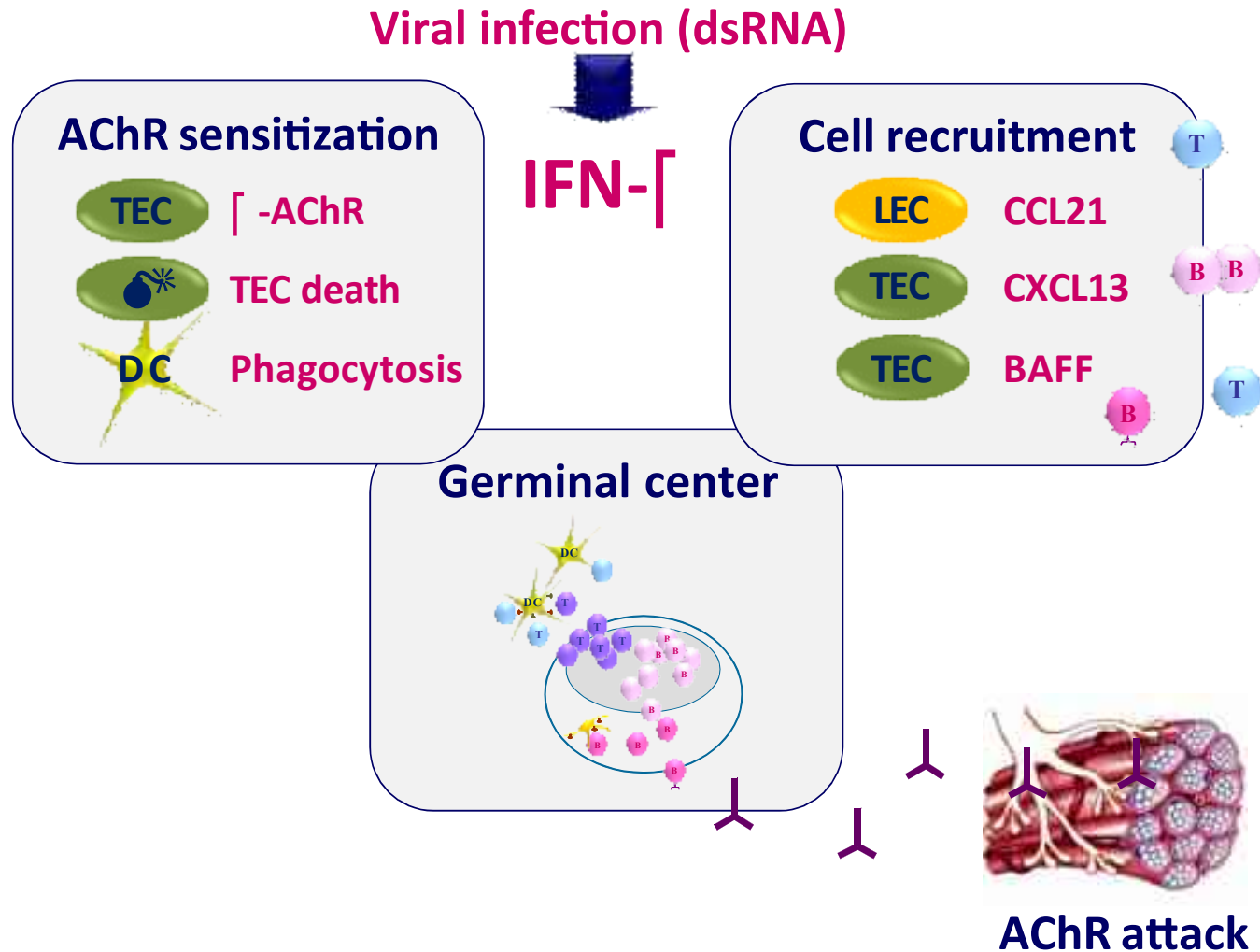
Anti-AChR Abs

AChR on muscle



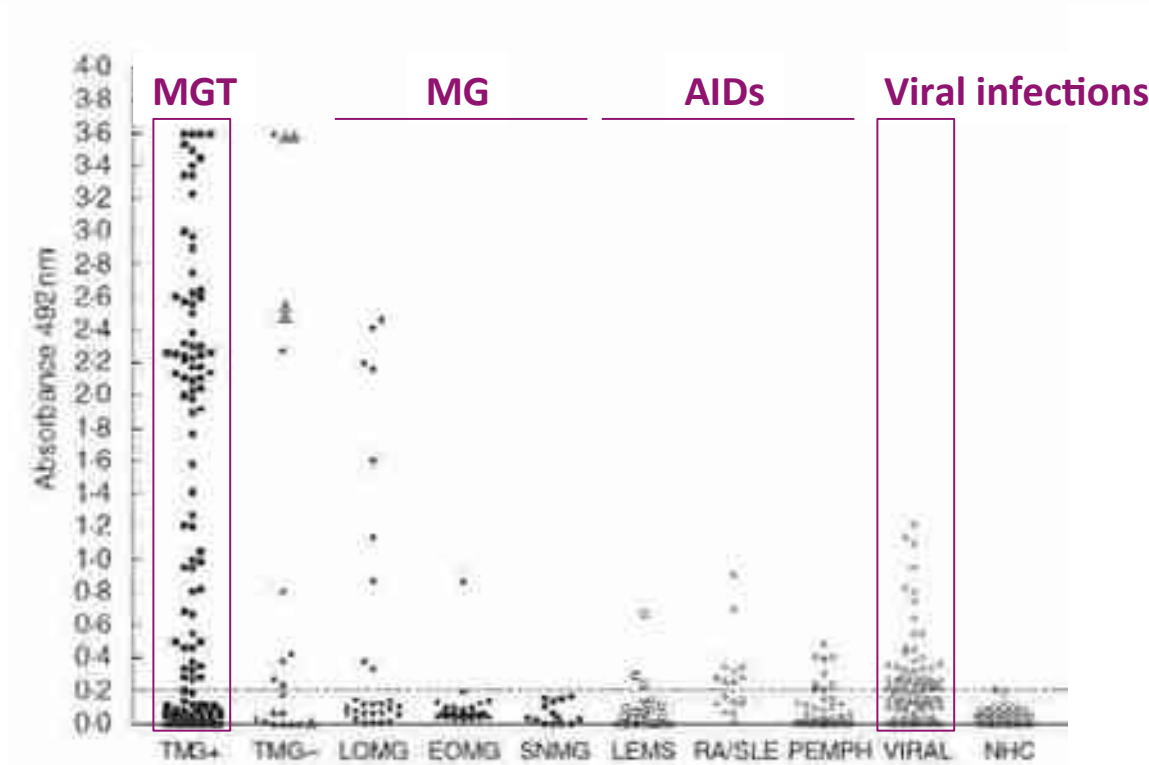
**Poly(I:C) triggered thymic  $\alpha$ -AChR expression through the release of IFN-I**

# IFN-I orchestrates thymic changes in EOMG



What about MG-associated thymoma ?

# Antibodies against IFN-I in MGT

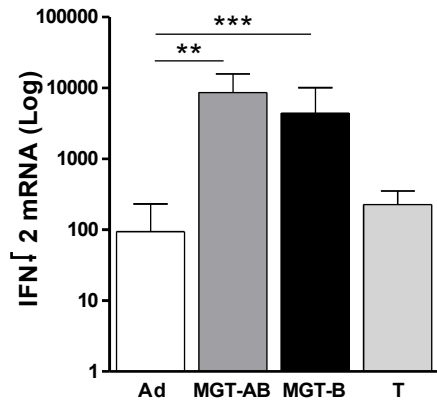


High levels of circulating anti-IFN $\alpha$ 2 in MGT

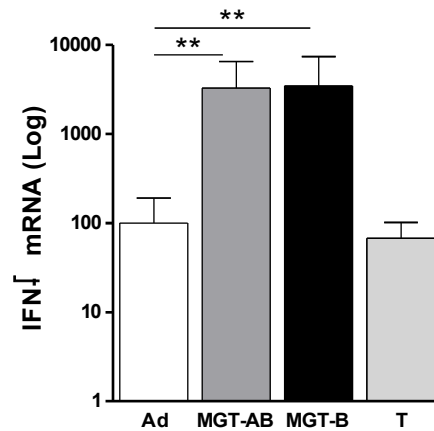


# IFN-I expression in the thymus of MGT

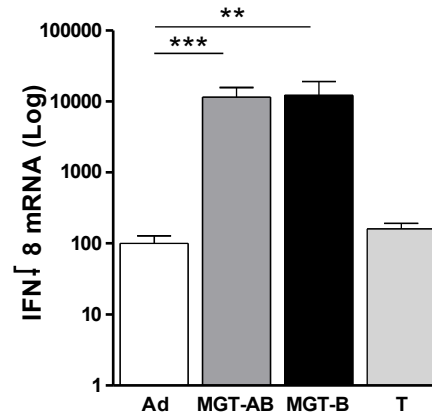
## IFN- $\alpha$ 2



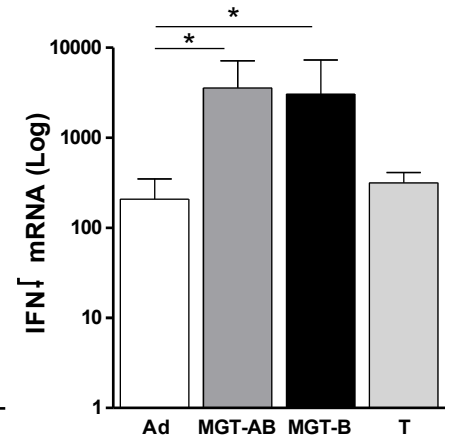
## IFN- $\omega$



## IFN- $\alpha$ 8

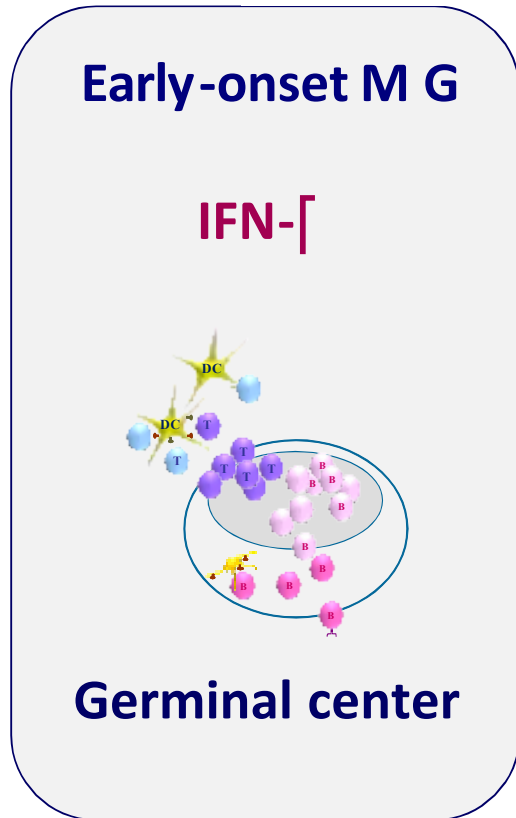


## IFN- $\beta$



**Specific overexpression of IFN-I subtypes in the thymus of MGT**  
**But not for thymoma without MG**

# Central role of IFN-I in anti-AChR response



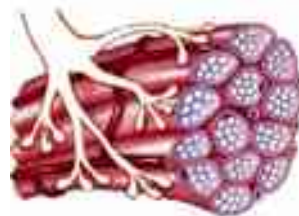
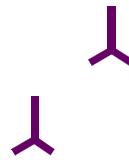
**Viral infection**



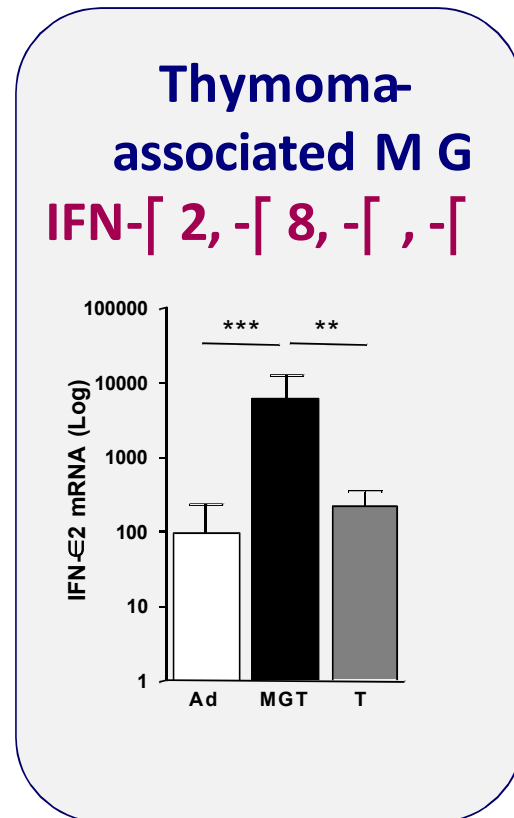
**IFN-I**



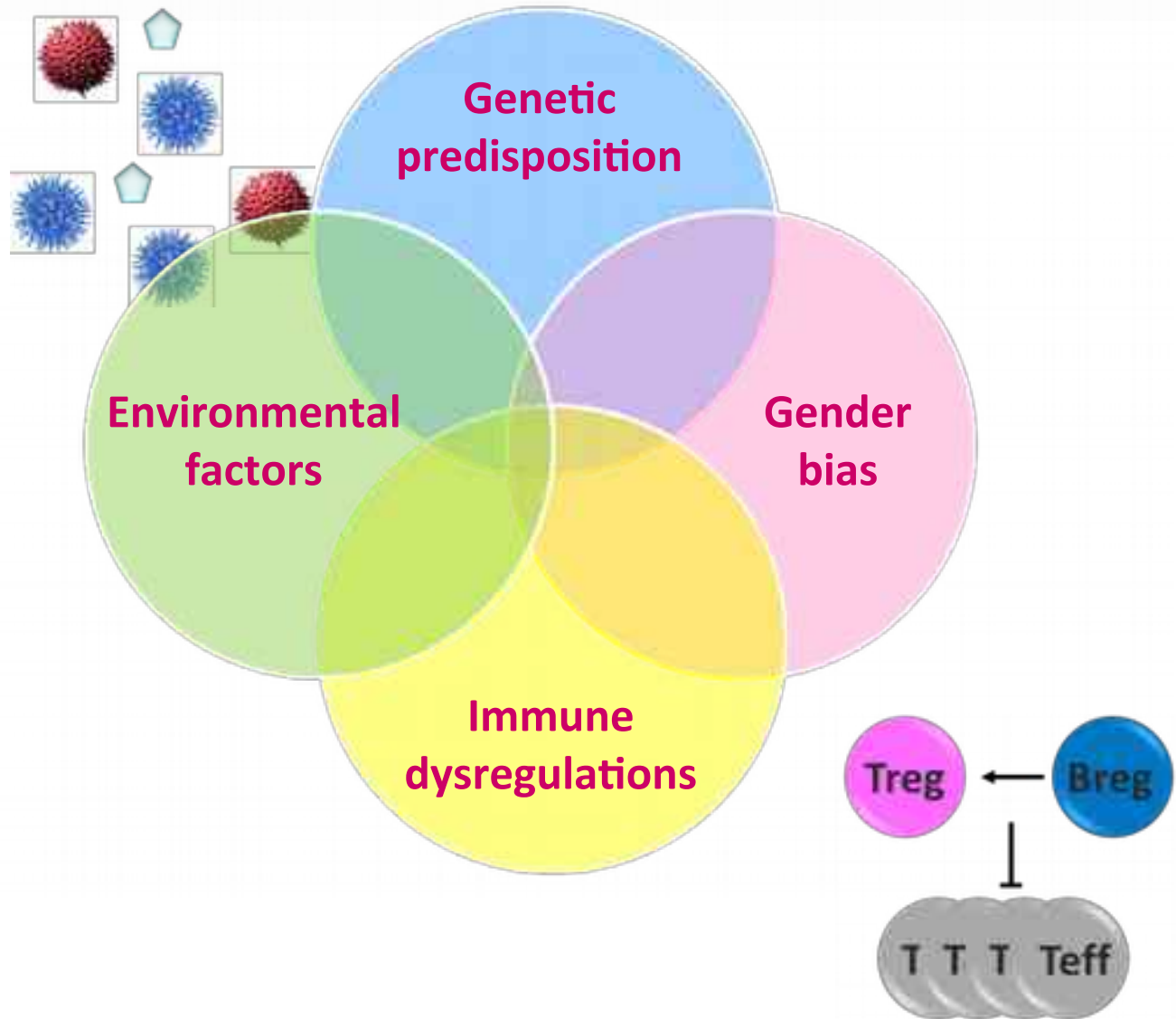
**IFN- $\gamma$ -AChR**



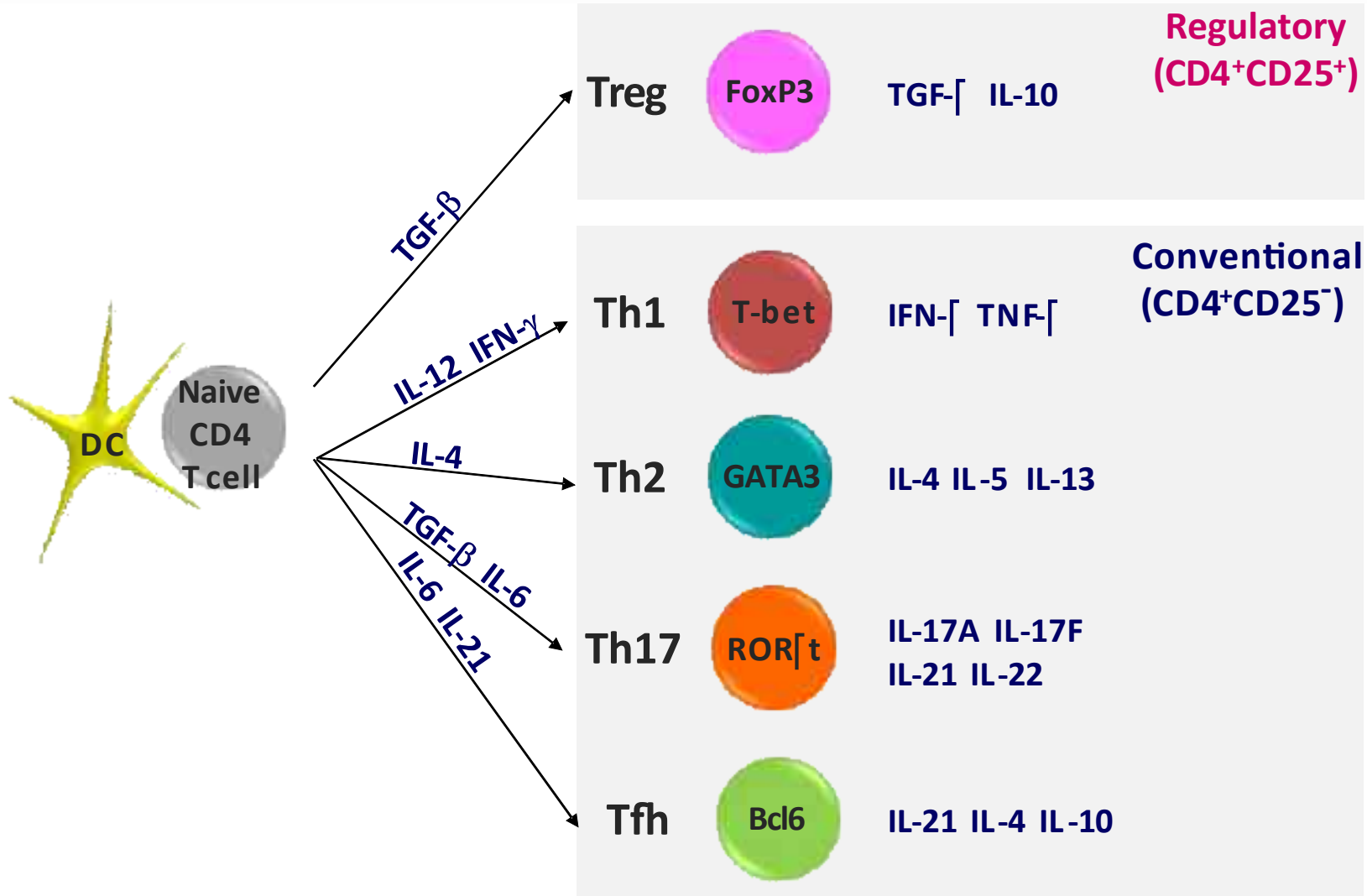
**AChR attack**



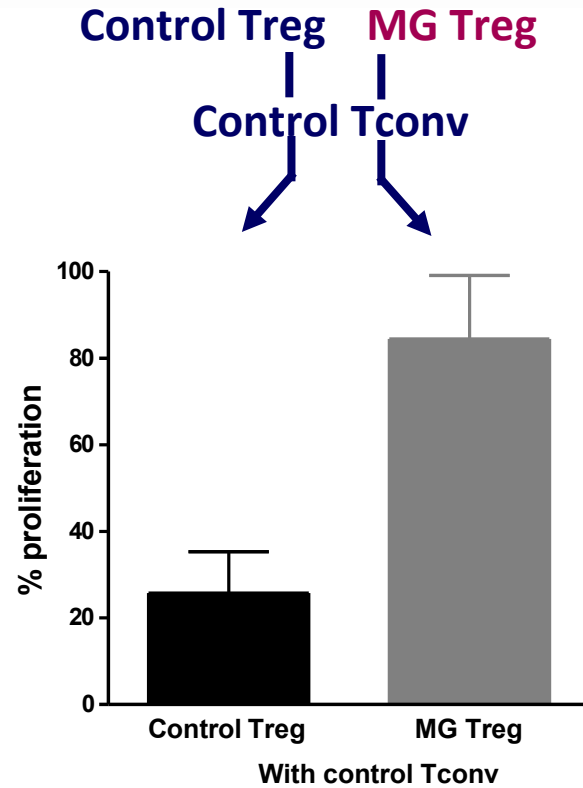
# Multifactorial disease



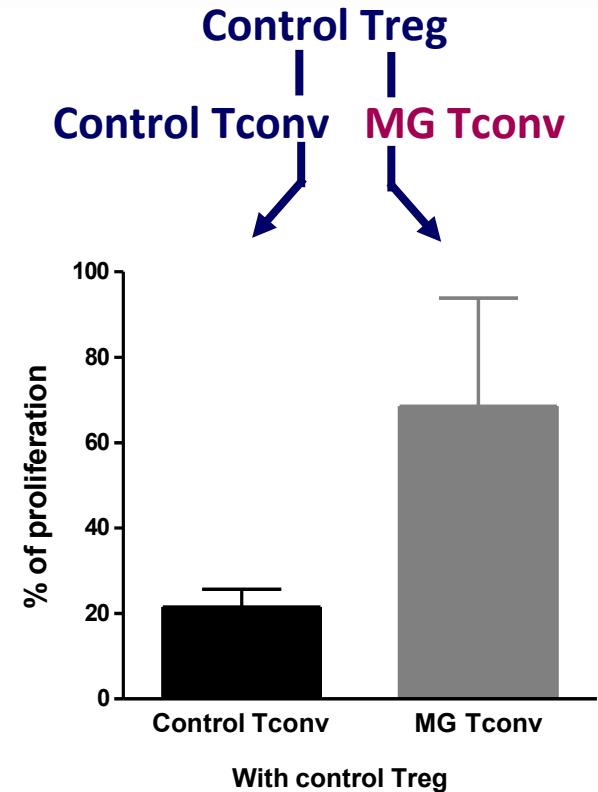
# Canonical representation of T cell subsets



# Defective Treg and Tconv cells in MG







**MG Treg are  
less suppressive**



**MG Tconv are  
more resistant to suppression**

# Why Treg and Tconv cells are defective in MG ?

Treg		TGF- $\gamma$ IL-10	Regulatory (CD4 <sup>+</sup> CD25 <sup>+</sup> )
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Th1		IFN- $\gamma$ TNF- $\gamma$	Conventional (CD4 <sup>+</sup> CD25 <sup>-</sup> )
Th2		IL-4 IL-5 IL-13	
Th17		IL-17A IL-17F IL-21 IL-22	
Tfh		IL-21 IL-4 IL-10	

## Microarray approach




**Genopolis**  
(Maria Foti)

- ✓ Human thymic tissues
- ✓ Control and MG donors (n=4-6)
- ✓ Cells separated by magnetic beads
  - ✓ CD4<sup>+</sup>CD25<sup>+</sup>: "TREG"
  - ✓ CD4<sup>+</sup>CD25<sup>-</sup>: "TCONV"

# Th1 and Th17 cytokine expression in MG Treg

**Treg**  **FoxP3** TGF- $\gamma$  IL-10 **Regulatory (CD4<sup>+</sup>CD25<sup>+</sup>)**

**Th1**  **T-bet** IFN- $\gamma$  TNF- $\gamma$  **Conventional (CD4<sup>+</sup>CD25<sup>-</sup>)**

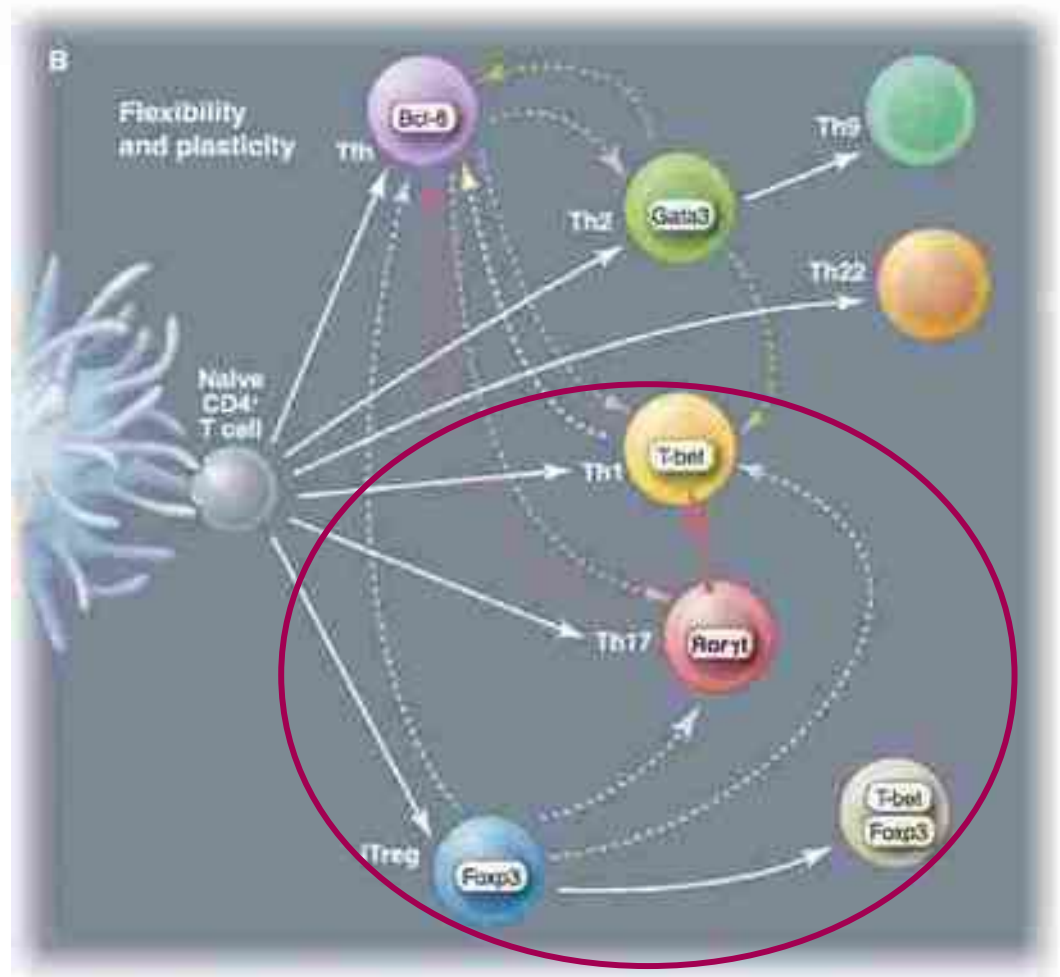
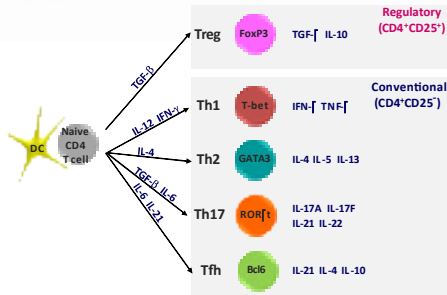
**Th2**  **GATA3** IL-4 IL-5 IL-13

**Th17**  **ROR $\gamma$ t** IL-17A IL-17F IL-21 IL-22

**Tfh**  **Bcl6** IL-21 IL-4 IL-10

Cytokine	Tconv	Treg
IL-1A	↑	
IL-17A		↑↑
IL-17B		↑↑
IL-17F		↑↑
IL-20		↑
IL-21	↑	↑↑
IL-22		↑↑
IL-26		↑↑
IL-32	↑↑	↑↑
IL-36A	↓↓	
IL-37		↑
TNF- $\alpha$	↑	↑↑
IFN- $\gamma$	↑	↑↑

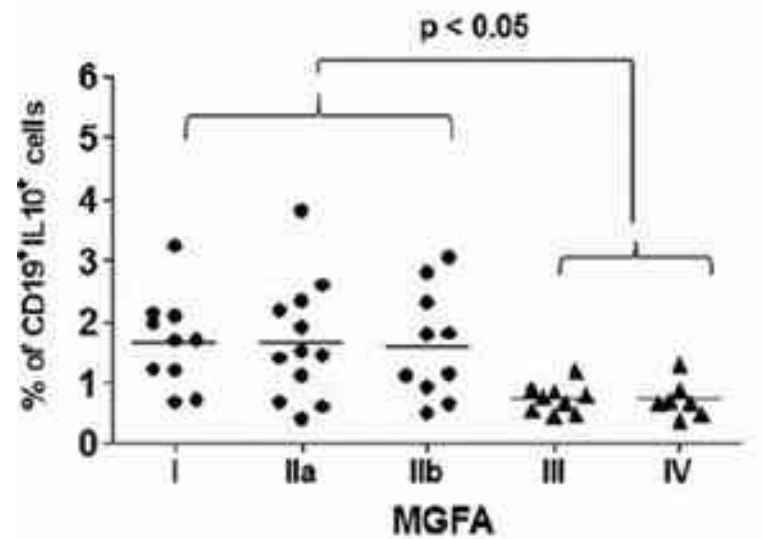
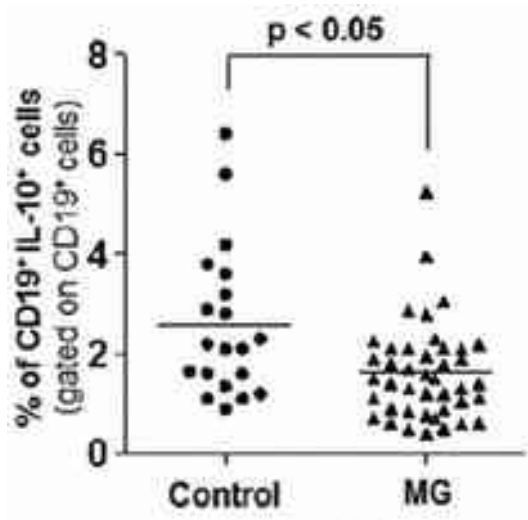
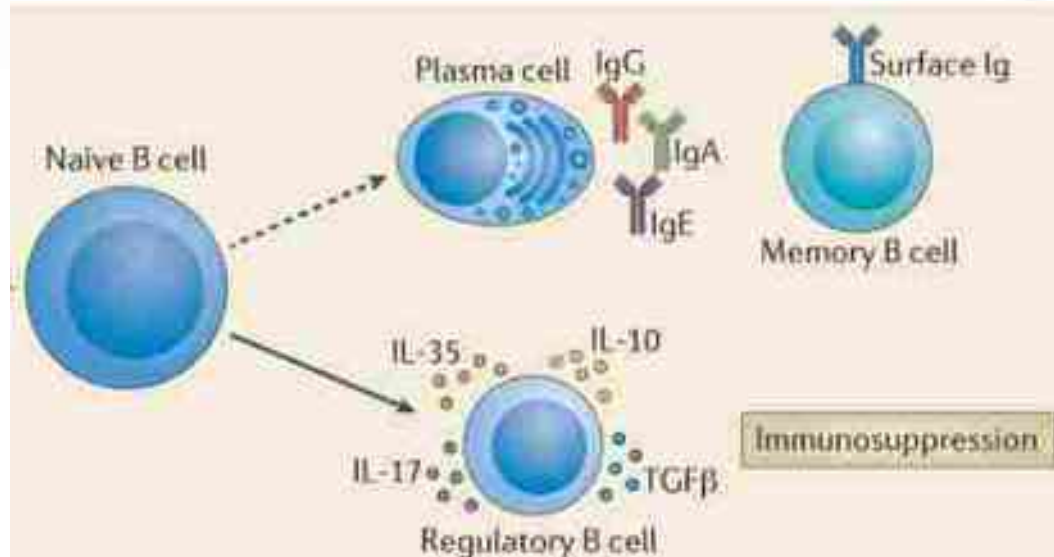
# Flexibility and plasticity of T cell subsets



**A switch of Treg toward Th17 phenotype in MG could explain the altered Treg suppressive function in MG**



# Regulatory B cells in MG



# Multifactorial disease

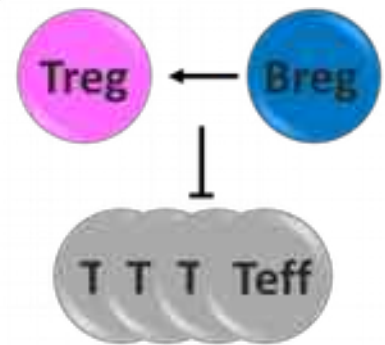
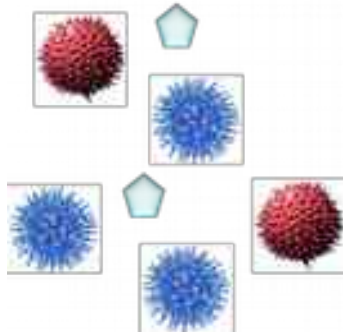
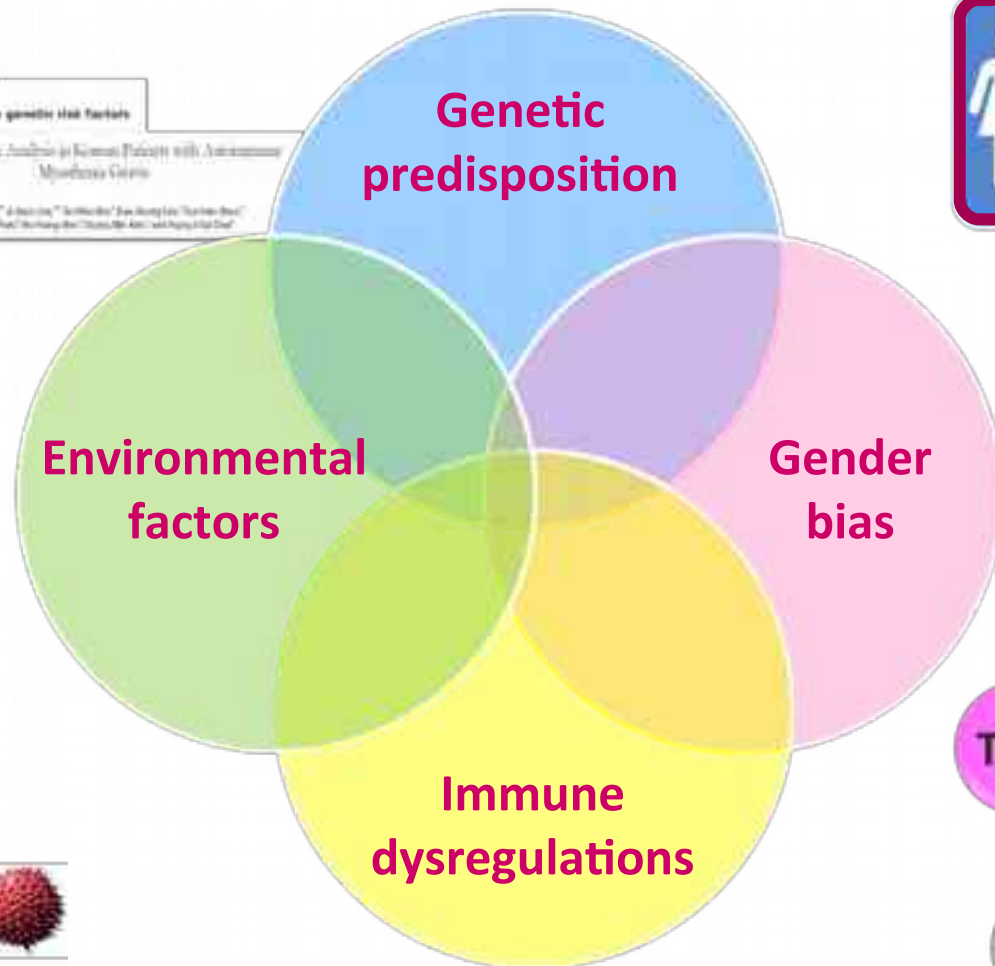
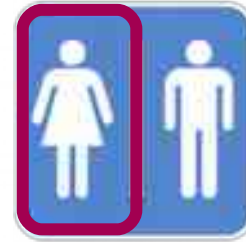
## Polymorphisms on HLA and other genes

Risk for Myasthenia Gravis Maps to a <sup>157</sup>Pro→Ala Change in TNIP1 and to Human Leukocyte Antigen-B\*08

SNP1 and SNF1, via NLR pathway, are genetic risk factors for myasthenia gravis

Whole Genome Analysis in German Patients with Antinuclear Myasthenia Gravis

## Female predisposition in EOMG



# Acknowledgments

## INSERM U974

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Angéline Gradolatto, Dany Nazzal  
Frédérique Truffault, Jacky Bismuth,

**Sonia Berrih-Aknin**

## Collaborators

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Hôpital Raymond-Poincaré (**T. SharShar**)

Hôpital Marie Lannelongue (**E. Fadel**)  
Institut Mutualiste Montsouris (**D. Gossot**)  
Hôpital Civile de Strasbourg (**N. Santelmo**)  
Hôpital COCHIN (**JF. Reignard**)

Fight-MG partners

