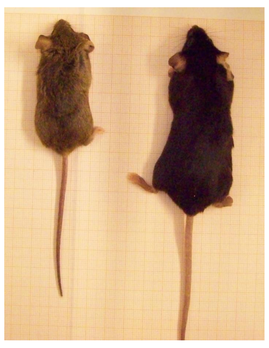
Severe deficiency of the somatotrope GHRH/GH/IGF-1 axis induces a dramatic susceptibility to *Streptococcus pneumoniae* infection

Khalil Farhat[1], Gwennaelle Bodart[1], Chantal Renard[1], Christophe Desmet[1], Michel Moutschen[2], Fréderic Baron[3], Yves Beguin[3], Roberto Salvatori[4], Vincent Geenen[1] & Henri Martens[1]

[1]*GIGA Research Institute, GIGA-I[3] Center of Immunoendoc rinology, University of Liege, Belgium*
[2]*Clinical Immunology and Infectiology, University Hospital of Liege, Belgium*
[3]*GIGA Research Hematology, University of Liege, Belgium*
[4]*Division of Endocrinology & Metabolism, Johns Hopkins University, Baltimore, USA*

The question of a physiological role of the somatotrope axis in immunity was reassessed in GHRH-deficient (*Ghrh*$^{-/-}$) mice, a new dwarf mouse model with a severe deficiency of the GHRH/GH/IGF-1 axis.

Ghrh$^{-/-}$ mice: severe deficiency the somatotrope GHRH/GH/IGF-1 axis



Hypothalamus → GHRH → Pituitary gland → GH → Liver → IGF1 → Bone, fat, muscles

Ghrh$^{-/-}$ mice: Phenotype

Alba, M. and R. Salvatori (2004) **Endocrinology** 145(9): 4134-4143.

- GH and IGF-1 deficiency
- Dwarf phenotype
- Normal bodily function
- Supplementation at different level of somatotropic axis

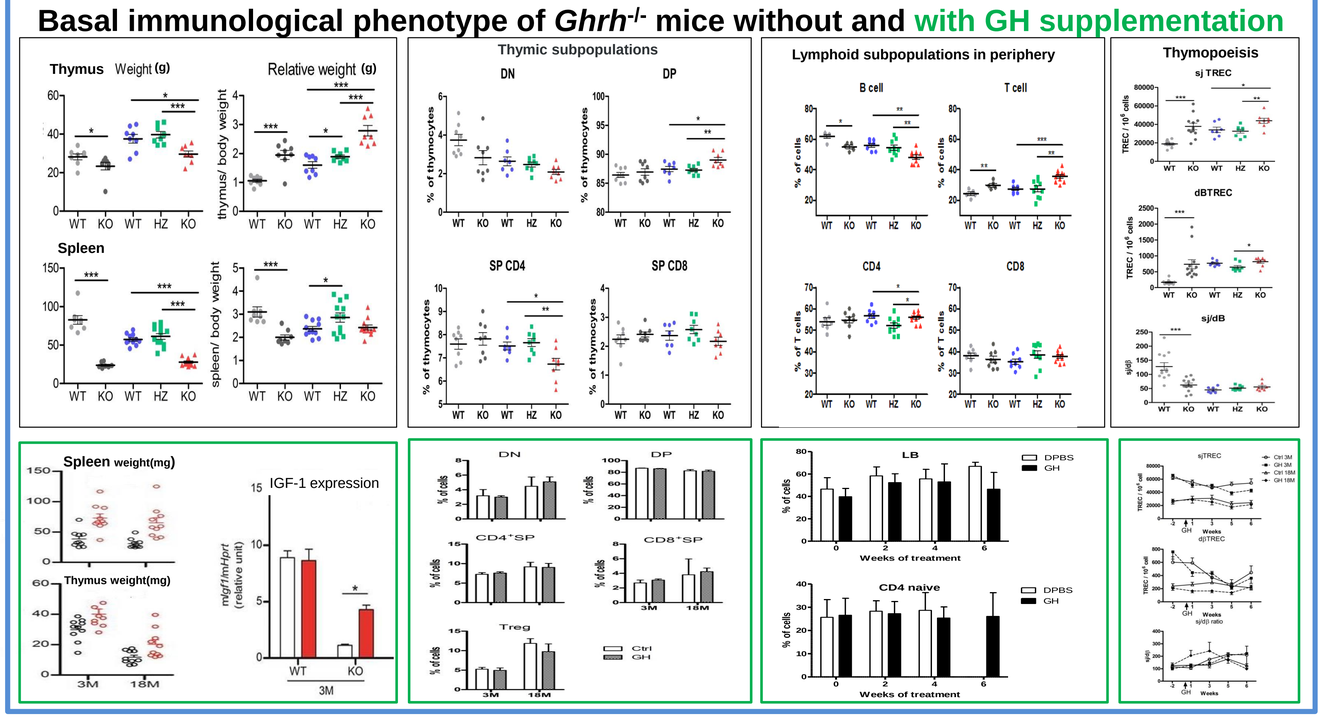
Ghrh$^{-/-}$ mice: Immune system

Shohreh, R., C. A. Pardo, F. Guaraldi, A. V. Schally and R. Salvatori (2011). **Endocrinology 152**(10): 3803-3810.

- Less prone to develop experimental autoimmune encephalomyelitis (EAE).
- GH administration but not GHRH restores the original susceptibility of EAE.

Pivotal role of GH on the regulation of different components of the immune system independent from GHRH

Basal immunological phenotype of *Ghrh*$^{-/-}$ mice without and with GH supplementation

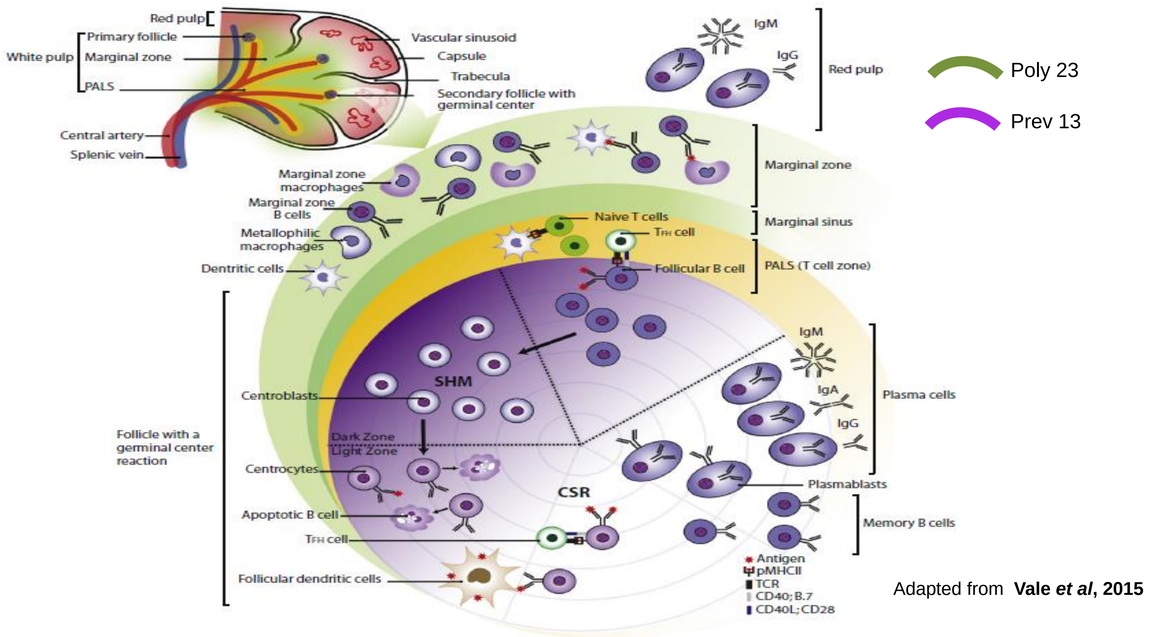


Characteristics of *Ghrh*$^{-/-}$ immune system in basal conditions: ↗ TREC, ↗ naive T cells, ↙↙ B cells.

Therefore, we investigated the B-dependent vaccine and immune responses of *Ghrh*$^{-/-}$ mice to 2 anti-pneumococcal vaccines and to a sublethal infection by *S.pneumoniae*.

Anti-pneumococcal vaccination

2 ways of vaccination against *S.pneumoniae*



Adapted from **Vale *et al*, 2015**

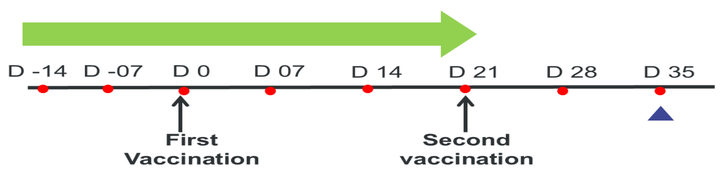
A- Polysaccharide vaccine (Poly 23):

- Primarily elicit Ab responses in the absence of T-cell help
- B-1 cells are envisioned as key players in this immune response along with marginal zone (MZ) B cells.

B- Conjugated pneumococcal vaccine (Prev-13)

- Conjugated to highly immunogenic cross-reactive material 197.
- Stimule FO B cells that engage in T-cell–B-cell cooperation, class-switching, yielding IgG antibodies of high affinity..

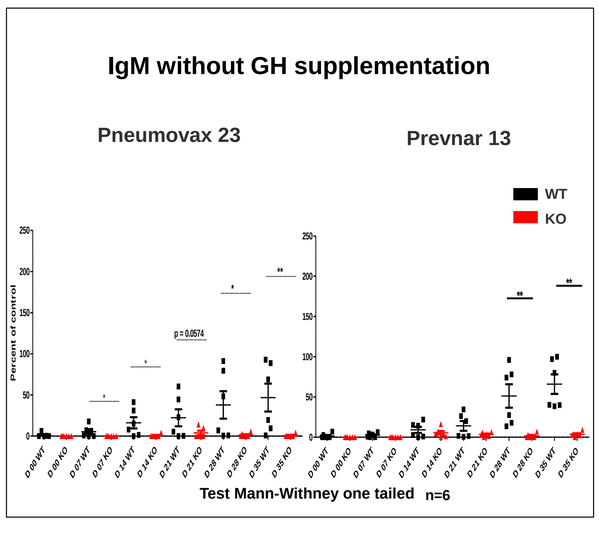
Experimental protocol



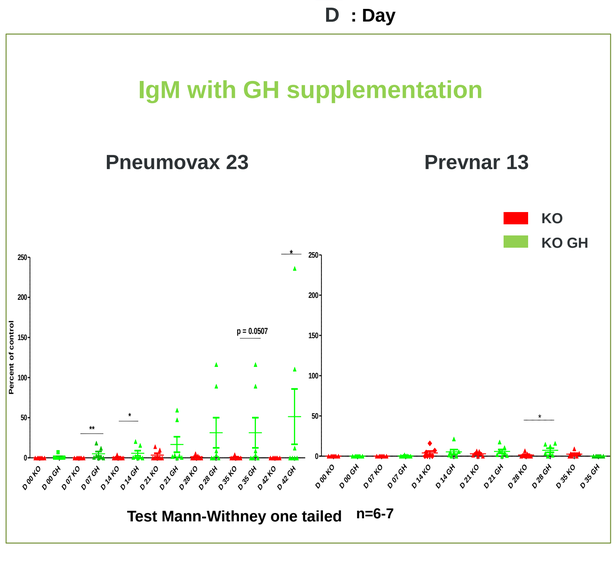
Age : 6-8 weeks
Vaccine administration: S.C
ELISA against CWPS1
GH supplementation : I.P

: GH supplementation
: Blood sample
: Sacrifice
D : Day

IgM without GH supplementation



IgM with GH supplementation



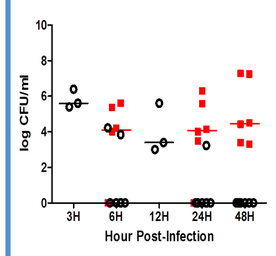
Ghrh$^{-/-}$ are unable to elicit vaccinal response to *S.pneumoniae* vaccines.
GH treatment restores vaccinal response of *Ghrh*$^{-/-}$ mice but only to Pneumovax 23.

Sublethal infection by *S.pneumoniae*

Experimental protocol

Results

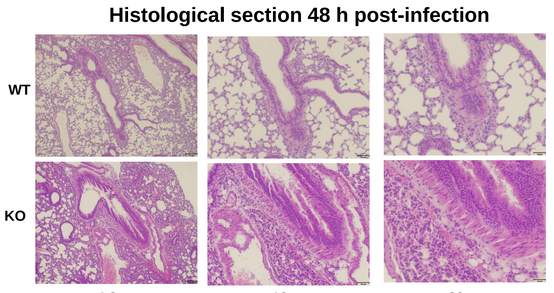
Bacterial load



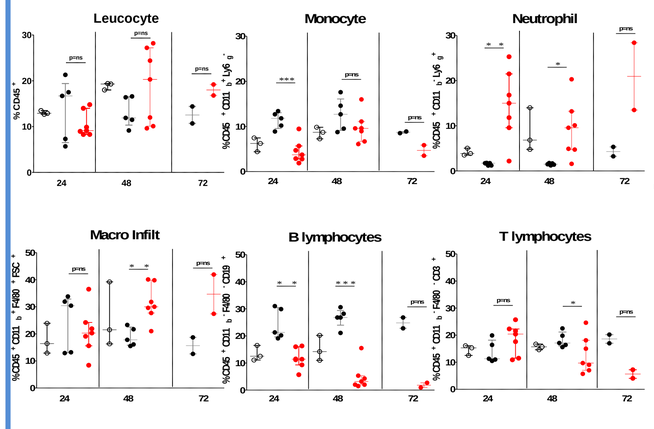
Bacteremia

	WT	Ghrh$^{-/-}$
6h	0/7	0/7
24h	0/13	8/12
48h	0/13	12/13
72h	0/3	3/3

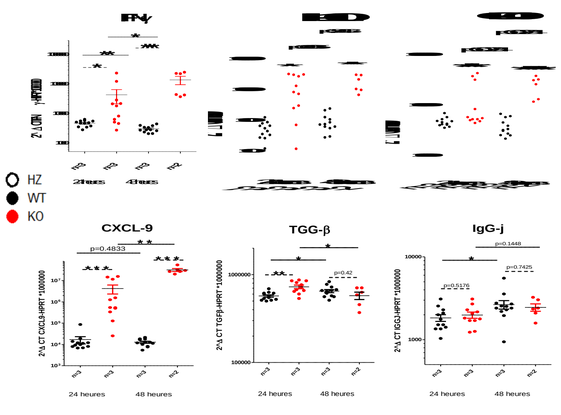
Histological section 48 h post-infection



Phenotype cells flow cytometry



In situ transcripts quantification



Unpaired t test with welch's correction one-tailed

Mann Whitney test Gaussian Approximation

***Ghrh*$^{-/-}$ mice develop *S.pneumoniae fatal* septicemia afer intranasal instillation while WT/HZ mice totally clear infection after 24 hours.**
There is also a marked decrease of B and T lymphocytes with high level of IFN-γ, IL-10, CD40 and CXCL-9 expression in the lungs of infected *Ghrh*$^{-/-}$ mice.

Supported by the Wallonia-Brussels Federation (Project Somasthym) and the ASSG (Beyrouth, Lebanon).