Growth hormone (GH) deficient mice with GHRH gene ablation are severely deficient in vaccine and immune responses against *Streptococcus pneumoniae*

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Abstract

In the general framework of intimate interactions between the neuroendocrine and immune systems, the precise impact of the hypothalamo-somatotrope GHRH/GH/IGF-1 axis upon the immune system is still highly debated. We have previously shown that mice with ablation of growth hormone (GH)-releasing hormone (GHRH) gene (*Ghrh*$^{-/-}$ mice) have normal thymus and T-cell development, but present a marked spleen atrophy and relative B-cell lymphopenia (1-3). Therefore, in this paper we have investigated vaccinal and anti-infectious responses of *Ghrh*$^{-/-}$ mice against *Streptococcus (S) pneumoniae*, a pathogen carrying T-independent antigens.
Ghrh$^{-/-}$ mice were unable to trigger production of specific IgM after vaccination with either native pneumococcal polysaccharides (PPS, PPV23) or protein-PPS conjugate (PCV13). Human GH (Genotorm) supplementation of *Ghrh*$^{-/-}$ mice partially restored IgM response to PPV23 vaccine but not to PCV13 suggesting that GH could exert a specific impact on the spleen marginal zone that is strongly implicated in T-independent response against pneumococcal polysaccharides.
After administration of a sublethal dose of *S. pneumoniae*, wild type (WT) completely cleared bacteria after 24h, as expected. In marked contrast, *Ghrh*$^{-/-}$ mice exhibited a dramatic susceptibility to *S. pneumoniae* infection with a time-dependent increase in lung bacterial load and a lethal bacteremia already after 24h. Lungs of infected *Ghrh*$^{-/-}$ mice were massively infiltrated by inflammatory macrophages and neutrophils, while lung B cells were markedly decreased. The inflammatory transcripts signature was also significantly elevated in *Ghrh*$^{-/-}$ mice. Important abnormalities of spleen cytoarchitecture and immune cell distribution were observed in *Ghrh*$^{-/-}$ mice both in basal and infected conditions.
This animal model unambiguously demonstrates that the hypothalamo-somatotrope GHRH/GH/IGF1 axis plays a vital and unsuspected role in vaccine and immunological defense against *S. pneumoniae*. In a translational perspective, these data indicate that spleen development and response to anti-pneumococcus vaccines should be cautiously monitored in children with isolated GH deficiency, from congenital and genetic origin in particular.

References

1. Alba M and Salvatori R. A mouse with targeted ablation of the growth hormone-releasing hormone gene: a new model of isolated growth hormone deficiency. *Endocrinology* (2004) **145**:4134-43. doi:10.1210/en.2004-0119
2. Shohreh R, Pardo CA, Guaraldi F, Schally AV and Salvatori R. GH, but not GHRH, plays a role in the development of experimental autoimmune encephalomyelitis. *Endocrinology* (2011) **152**:3803-10. doi:en.2011-1317 [pii] 10.1210/en.2011-1317
3. Bodart G*, Farhat K*, Renard-Charlet C, Becker G, Plenevaux A, Salvatori R., et al. The severe deficiency of the somatotrope GH-releasing

hormone/Growth hormone/Insulin-like growth factor 1 axis of *Ghr*h$^{-/-}$ mice is associated with an important splenic atrophy and relative B lymphopenia. *Front. Endocrinol.* (2018) **9**:296. doi: 10.3389/fendo.2018.00296
4. Farhat, K*, Bodart, G*, Renard-Charlet C, Desmet C, Moutschen M, Beguin, Y et al. Growth hormone (GH) deficient mice with GHRH gene ablation are severely deficient in vaccine and immune responses against *Streptococcus pneumoniae*. *Front. Immunol.*, under revision
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