HORMONES, NEUROTRANSMITTERS, AND T-CELL DEVELOPMENT IN HEALTH AND DISEASE

EDITED BY: Wilson Savino, Daniella Areas Mendes-da-Cruz, Ana Rosa Pérez and Vincent Geenen

PUBLISHED IN: Frontiers in Endocrinology

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88963-026-4
DOI 10.3389/978-2-88963-026-4

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

HORMONES, NEUROTRANSMITTERS, AND T-CELL DEVELOPMENT IN HEALTH AND DISEASE

Topic Editors:
Wilson Savino, Oswaldo Cruz Foundation (Fiocruz), Brazil
Daniella Areas Mendes-da-Cruz, Oswaldo Cruz Foundation (Fiocruz), Brazil
Ana Rosa Pérez, National Council for Scientific and Technical Research (CONICET), Argentina
Vincent Geenen, University of Liège, Belgium

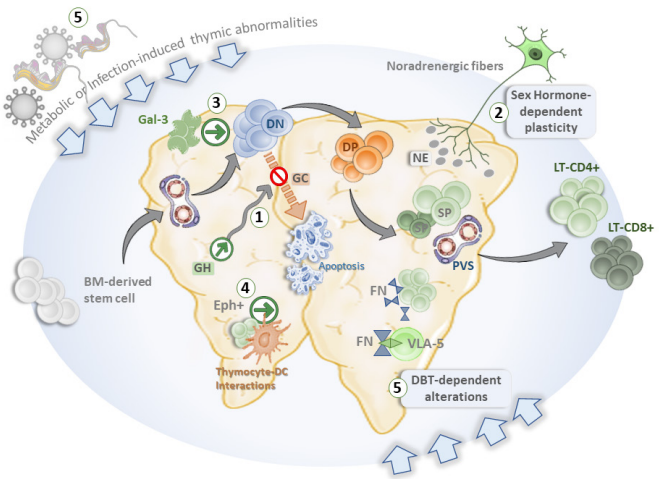

Image: Ana Rosa Perez

Thymus physiology and T-cell homeostasis are controlled by hormones, neurotransmitters, cytokines and other factors that modulate stromal-cell interactions, influence thymocyte development and selection processes, survival and migration, between others.

In the context of this Research Topic on "Hormones, Neurotransmitters, and T cell development in Health and Disease", authors discuss the control of thymus physiology by glucocorticoids (GC), growth hormone (GH) and sex hormones, norepinephrine (NE) and other molecules that seem impact upon thymocyte/microenvironmental interactions, like galectin-3 (Gal-3) ephrins (Eph), extracellular matrix proteins and integrins (like VLA-5). Moreover, some of them draw attention to about how diverse maturation steps and/or the interactions between stromal and thymocytes can be affected in pathological states like diabetes or infections.

As shown schematically in the figure, this topic highlight the following notions:
1) GH shows counterregulatory effects against GC rather than influence directly T cell homeostasis;
2) Interactions between sex hormones and noradrenergic secretion may influence thymus homeostasis and involution;
3) Gal-3 are crucial to thymocyte-stromal cell interactions and influence thymic architecture. Moreover, Gal-3 seem to be involved in the regulation of steroidogenic pathway;
4) Ephrins are crucial to assembly the thymic connections between thymocytes and the epithelial network, but have a relative importance in supporting normal thymopoyesis;
5) Pathologic situations like diabetes, or infectious diseases caused by parasites or bacteria alters the normal development of T-lymphocytes and might influence tolerance process. DN: double-negative (CD4-CD8) thymocytes; DP: double-positive (CD4+CD8+) thymocytes; SP: simple-positive thymocytes; LT: T lymphocyte; FN: fibronectin; PVS: peri-vascular space; BM: bone marrow; DBT: diabetes; Eph: Ephrins.

Citation: Savino, W., Mendes-da-Cruz, D. A., Pérez, A. R., Geenen, V., eds. (2019). Hormones, Neurotransmitters, and T-Cell Development in Health and Disease. Lausanne: Frontiers Media. doi: 10.3389/978-2-88963-026-4

Table of Contents

frontiers in Endocrinology

EDITORIAL
published: 11 July 2019
doi: 10.3389/fendo.2019.00454

Editorial: Hormones, Neurotransmitters, and T-Cell Development in Health and Disease

Ana Rosa Pérez[1]*, *Daniella Areas Mendes-da-Cruz*[2,3], *Vincent Geenen*[4] and *Wilson Savino*[2,3]

[1] Institute of Clinical and Experimental Immunology of Rosario, (IDICER-National Scientific and Technical Research Council), Rosario, Argentina, [2] Laboratory on Thymus Research, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, [3] National Institute of Science and Technology on Neuroimmunomodulation, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, [4] Department of Biomedical and Preclinical Sciences, GIGA-I3 Center of Immunoendocrinology, GIGA Research Institute, University of Liège, Liège, Belgium

Keywords: thymus, hormones, neurottransmitters, T lymhocytes, thymopoiesis

Editorial on the Research Topic

Hormones, Neurotransmitters, and T-Cell Development in Health and Disease

Thymus physiology, T-cell development, and peripheral T-cell homeostasis are controlled by a large variety of soluble molecules and their cognate receptors, targeting both the lymphoid and non-lymphoid compartments. Hormones, neurotransmitters, and cytokines influence the functions of distinct microenvironmental cells, including their maturation, survival, and antigen presentation. Additionally, they affect thymocyte survival, migration and selection, thus shaping the pool of mature T-cells in the periphery. Importantly, some of these circuits can be affected in pathological states.

Beneficial effects of the somatotrope axis on thymopoiesis have been extensively reported. Most of the data derive from studies carried out in mouse models with multiple pituitary deficiencies (i.e., lacking GH, PRL, and thyrotropic hormones), making it difficult to identify the real effect of GH on T cell homeostasis. Here, Bodart et al. show a series of studies carried out in GhrhKO mice, revealing the absence of thymic involution (in terms of relative weight or cellularity), accompanied only by minor changes in the proportions of thymocyte subsets. Authors also obtained data compatible with a faster commitment of double negative thymocytes in the thymopoietic process accompanied by an increased thymic output of naïve T cells, this later observation being consistent with a reduction of central memory T cells in secondary lymph organs. Taken together, these findings point out that the integrity of the GHRH/GH/IGF-1 axis is not required for thymocyte and peripheral T cell homeostasis in basal conditions, although it can influence the splenic B cell compartment. Overall, these data suggest that GH beneficial effects upon thymus homeostasis may be rather related with the positive counter regulatory effects of GH and PRL against the stress caused by glucocorticoids.

Additionally, it is known that with aging, thymic involution is accompanied by a diminution the thymopoietic capacity. The thymus is highly innervated by noradrenergic fibers and there is also a local production of norepinephrine, and both thymocytes and microenvironmental cells express adrenergic receptors. Leposavić and Pilipović examined the influence of sex steroids upon thymopoiesis during both perinatal/peripubertal evolving periods. The authors show that such hormones alter the adrenergic thymic microenvironment, although the mechanisms are still unknown. Yet, the available data insinuate that pharmacological handling of noradrenergic effects upon the thymus may improve the deleterious effects of aging on thymopoiesis.

Glucocorticoids can act on practically all types of cells, particularly T cells, showing to have upon these cells important immunosuppressive and anti-inflammatory effects, but also affect their

OPEN ACCESS

Edited and reviewed by:
Hubert Vaudry,
Université de Rouen, France

***Correspondence:**
Ana Rosa Pérez
perez_anarosa@yahoo.com.ar;
perez@idicer-conicet.gob.ar

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: *22 May 2019*
Accepted: *21 June 2019*
Published: *11 July 2019*

Citation:
Pérez AR, Mendes-da-Cruz DA, Geenen V and Savino W (2019) Editorial: Hormones, Neurotransmitters, and T-Cell Development in Health and Disease. Front. Endocrinol. 10:454. doi: 10.3389/fendo.2019.00454

phenotype, secretion profile, as well as survival and migratory properties. Here, Liberman et al. explored deeply the complexity of glucocorticoid effects upon immune cells (particularly on developing T cells), and the glucocorticoid receptor-mediated regulation. Importantly, authors also discuss how glucocorticoids induce both paradoxical anti- and pro-inflammatory responses, particularly in certain areas of the brain, in order to develop more effective treatments and avoid side effects, like toxicity and drug resistance.

Thymocyte-microenvironmental cell interactions are known to be critical for thymus homeostasis. The studies made by Oliveira-de-Abreu et al. strengthens the notion that changes in thymocyte/microenvironmental cell contact cause profound thymic alterations. For example, in the absence of Galectin-3 (Gal-3; a molecule abundantly expressed in the thymus that acts as a de-adhesive factor), the thymus showed abnormalities in developing T-cell number, proliferation, and death, with proportional enlargement of the DN1 compartment and a severe disorganization of the epithelial network. These findings reveal that Gal-3 is not only relevant for thymocyte homeostasis, but also to the maintenance of thymic architecture. Strikingly, glucocorticoid secretion is enhanced in the absence of Gal-3, possibly by a rise of both the adrenal and thymic steroidogenic machinery. How Gal-3 regulates glucocorticoid secretion has not yet been established and demands further studies. In contrast, Muñoz et al. adduce that not necessarily a disorder of the thymic epithelial network results in altered T development. Previous studies of the group showed that Ephrins (Eph) and their receptors seem to be relevant in both temporal and topologic thymocyte/thymic epithelial cell (TEC) interactions, modulating intrathymic thymocyte migration. Mice deficient in EphB2 and EphB3 showed severe abnormalities in TEC morphology, but relatively normal thymocyte subpopulations and immunocompetence. Here, the authors provide evidence supporting the notion that, regardless of Eph-deficient mice exhibited an altered epithelium, their TECs could express all necessary molecules to support thymocyte development and differentiation.

Two additional articles focused on diabetes, discussing possible relationships between thymic alterations, the development of autoreactive T lymphocytes and the establishment of abnormal immunoendocrine and metabolic profiles. Mendes-da-Cruz et al. discuss thymic disturbances observed in a well-established model of type-1 diabetes, as are NOD (non-obese diabetic) mice. A hallmark of the NOD thymus is the presence of enriched areas in simple-positive $CD4^+$ and $CD8^+$ thymocytes, possibly secondary to a defective expression of the fibronectin receptor VLA-5. It is well-known that diminished insulin-derived peptide expression in the thymus may favor the breakdown of insulin tolerance. Nevertheless, recent data show that expression of several miRNA is altered in the thymus of NOD mice, suggesting that some of them are involved in the mechanism underlying the generation of autoreactive cells. In addition, Andreone et al. make an insightful review of how hormones and neurotransmitters influence diverse T compartments under metabolic imbalance triggered by diabetes and their role in aggravating the disease.

Last, D'Attilio et al. and Pérez et al. review the immunoendocrine alterations reported during tuberculosis and Chagas disease, respectively. Although the real impact at the thymic level of both diseases in humans requires even more studies, data from experimental models strongly suggest that thymic abnormalities that take place in response to *Micobacterium tuberculosis* and *Trypanosoma cruzi* could act as contributing factors to pathology.

In the last years our understanding of the intricate network of neurotransmitters, hormones, cytokines and other molecules that play a major role in the regulation of T cell development and biological functions has increased enormously, but substantial challenges remain to provide a more comprehensive picture. The articles presented in this Research Topic reveal new evidence that illustrates the complex circuitries affecting the thymus and T cell homeostasis in both health and disease and point to further directions of future research.

AUTHOR CONTRIBUTIONS

AP wrote the manuscript with input from all authors, who made substantial contributions to the Editorial and approved it for publication.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 06 June 2018
doi: 10.3389/fendo.2018.00296

The Severe Deficiency of the Somatotrope GH-Releasing Hormone/Growth Hormone/Insulin-Like Growth Factor 1 Axis of *Ghrh*$^{-/-}$ Mice Is Associated With an Important Splenic Atrophy and Relative B Lymphopenia

Gwennaelle Bodart[1†], Khalil Farhat[1†], Chantal Renard-Charlet[1], Guillaume Becker[2], Alain Plenevaux[2], Roberto Salvatori[3], Vincent Geenen[1†] and Henri Martens[1†]*

[1] GIGA-I[3] Center of Immunoendocrinology, GIGA Research Institute, University of Liege, Liège, Belgium, [2] Cyclotron Research Center, University of Liege, Liège, Belgium, [3] Division of Endocrinology, Diabetes, and Metabolism, Department of Medicine, The Johns Hopkins University, Baltimore, MD, United States

A debate is still open about the precise control exerted by the somatotrope GH-releasing hormone (GHRH)/growth hormone (GH)/insulin-like growth factor 1 axis on the immune system. The objective of this study was to directly address this question through the use of *Ghrh*$^{-/-}$ mice that exhibit a severe deficiency of their somatotrope axis. After control backcross studies and normalization for the reduced global weight of transgenic mice, no difference in weight and cellularity of the thymus was observed in *Ghrh*$^{-/-}$ mice when compared with C57BL/6 wild-type (WT) control mice. Similarly, no significant change was observed in frequency and number of thymic T cell subsets. In the periphery, *Ghrh*$^{-/-}$ mice exhibited an increase in T cell proportion associated with a higher frequency of sjTREC and naïve T cells. However, all *Ghrh*$^{-/-}$ mice displayed an absolute and relative splenic atrophy, in parallel with a decrease in B cell percentage. GH supplementation of transgenic mice for 6 weeks induced a significant increase in their global as well as absolute and relative splenic weight. Interestingly, the classical thymus involution following dexamethasone administration was shown to recover in WT mice more quickly than in mutant mice. Altogether, these data show that the severe somatotrope deficiency of *Ghrh*$^{-/-}$ mice essentially impacts the spleen and B compartment of the adaptive immune system, while it only marginally affects thymic function and T cell development.

Keywords: somatotrope axis, GH-releasing hormone, growth hormone, insulin-like growth factor 1, thymus, developmental immunology

Abbreviations: LN, lymph node; WT, wild-type; FSC, forward scatter; SSC, side scatter; TREC, T-cell receptor excision circle; Sj, signal joint; RT-qPCR, real-time quantitative PCR; DN, double-negative; DP, double-positive; SP, single-positive; TCM, central memory T cell; TEM, effector memory T cell; GC, glucocorticoid.

OPEN ACCESS

Edited by:
Lee E. Eiden, National Institutes of Health (NIH), United States

Reviewed by:
Frederik Jacobus Steyn, The University of Queensland, Australia
Laurent Kappeler, Institut National de la Santé et de la Recherche Médicale (INSERM), France

****Correspondence:***
Vincent Geenen
vgeenen@uliege.be

[†]Equal first and senior authors.

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: *05 February 2018*
Accepted: *17 May 2018*
Published: *06 June 2018*

Citation:
Bodart G, Farhat K, Renard-Charlet C, Becker G, Plenevaux A, Salvatori R, Geenen V and Martens H (2018) The Severe Deficiency of the Somatotrope GH-Releasing Hormone/Growth Hormone/Insulin-Like Growth Factor 1 Axis of Ghrh$^{-/-}$ Mice Is Associated With an Important Splenic Atrophy and Relative B Lymphopenia. Front. Endocrinol. 9:296. doi: 10.3389/fendo.2018.00296

INTRODUCTION

Integrated homeostasis of living organisms closely depends on the intimate crosstalk between the major systems of cell-to-cell signaling, the immune, nervous, and endocrine systems. Growth hormone (GH) secretion by somatotroph cells of the antehypophysis is stimulated by the hypothalamic GH-releasing hormone (GHRH) and its biological effects are mediated by GH receptor, a member of the class I cytokine receptor superfamily (1). Most of the metabolic effects of GH on peripheral tissues are direct, while the growth-promoting action is mainly indirect, through the endocrine and paracrine–autocrine insulin-like growth factor 1 (IGF1).

Already in 1930, Philip E. Smith observed that hypophysectomy in rats induced a severe thymic involution (1). Numerous studies in two mouse models of pituitary deficiency, the Snell–Bagg and Ames Dwarf mice—mutated in Pit1 and Prop1 transcription factors, respectively—confirmed the regulation of the immune system by pituitary hormones since those mice exhibited a thymo-dependent immune deficiency that could be prevented or reversed by administration of GH and thyroxine (2–4). This was reinforced by the observation of aged-atrophic thymus rejuvenation by GH-producing pituitary adenoma cells (5). The effects mediated by GH and IGF1 on immune cells have been extensively reviewed (6, 7).

Positive effects of the somatotrope axis on human thymopoiesis have also been reported. Treatment of HIV+ patients with GH in combination with highly active antiretroviral therapy increases thymic volume, $CD4^+$ naïve T cell number, and thymopoiesis evaluated by the frequency of signal joint (sj) T-cell receptor excision circles (TRECs) (8). One-month withdrawal of GH in patients with adult GH deficiency diminished thymic output of new T cells and intrathymic T-cell proliferation, as evidenced by the decrease in sjTREC frequency and sj/DJβTREC ratio, respectively, and these parameters were restored 1 month after GH resumption (9). Given their important beneficial effects on thymopoiesis, GH and IGF1 are more and more considered for their use as immunomodulatory agents in acquired immune deficiencies such as in HIV infection and aging.

Despite this large experimental evidence, the role of GH in immunologic regulation remains controversial and is still discussed. In contradiction with previous studies, some authors observed a normal thymus weight and number of T-cell subsets in Snell–Bagg dwarf and in *lit/lit* (GHRH receptor-deficient mice) mice (10–12). Dorshkind and Horseman (13) concluded that the immunomodulatory properties of the pituitary hormones GH and prolactin essentially result from their ability of counteracting the negative effects mediated by stress-induced glucocorticoids (GCs) upon the immune system.

The objective of this study was to investigate the basal thymic and immunologic phenotype of a transgenic mouse model with a severe deficiency of the somatotrope axis resulting from a targeted disruption of the GHRH gene (14). *Ghrh*$^{-/-}$ mice exhibit a dwarf phenotype due to a severe GH and IGF-1 deficiency that can be supplemented either at the hypothalamic, hypophysial, or peripheral levels of the somatotrope axis (15–17).

MATERIALS AND METHODS

Mice

*Ghrh*KO mouse strain (C57BL/6 background) was previously developed by one of us as previously described (14). Wild-type (WT) C57BL/6 mice were obtained from Charles River Laboratories. Both strains were kept and bred at the animal facility of the University of Liège. All animals were bred in ventilated cages at the Central Animal Facility of Liege University (GLP certified, LA.2610359) of the University of Liège with a 12-h light/12-h dark cycle with food and water *ad libitum*. We performed a backcross between those two strains, to obtain animals with completely identical genetic background. Briefly, *Ghrh*KO and C57Bl/6 mice were bred together to obtain a F1 generation of heterozygous (HZ) animals. F1 animals were mated together and gave rise to F2 mice with *Ghrh*$^{+/+}$ (called WT in the text), *Ghrh*$^{+/-}$ (HZb), and *Ghrh*$^{-/-}$ (called *Ghrh*KO in the text) animals (respectively, 25, 50, and 25% proportion expected). Mouse genotype was identified phenotypically: original *Ghrh*$^{-/-}$ mice have agouti color, a dominant trait, where agouti gene is located near the *Ghrh* mutated gene, so they are transmitted together. Therefore, WT backcrossed F2 mice are black and normal-sized; HZ backcrossed animals are agouti and normal-sized and KO backcrossed mice are agouti and dwarf. Normal-sized and dwarf mice were separated at least 4 weeks before any experiment. Male and female mice of 3, 6, or 18 months were used for the characterization experiments, and 3 or 18 months for the GH supplementation experiments. All the experiments were conducted with approval of the Institutional Animal Care and Use Committee of the University of Liège (permit no. 1305) in strict accordance with the guidelines for the care and use animals set out by the European Union.

Tissue and Cell Preparation

Mice were euthanized by i.p. injection of ketamine (100 mg/kg)–xylazine (10 mg/kg) followed by cardiac puncture. Thymus, spleen, and inguinal lymph nodes (LNs) were removed and weighted. A piece of liver was also removed when needed for IGF-1 quantification. PBMC were isolated from whole blood by centrifugation in Lympholyte®-Mammal density separation medium (Cedarlane), according to the manufacturer's instructions. Single-cell suspensions were obtained from the thymus, spleen, and LN by mechanical disruption, followed by two washing steps at 500 *g* for 5 min in Dulbecco's phosphate-buffered saline (DPBS, Lonza). An additional RBC lysing step was performed to eliminate RBC from splenic cell suspension by incubating 5 min in 1 ml of RBC Lysis Buffer Hybri-Max (Sigma-Aldrich) before a final washing step. Cell suspensions were then passed through 70-µm Nylon cell strainer (Falcon) and diluted to the appropriated concentration in DPBS.

Flow Cytometry

For analysis of lymphocyte subpopulations in thymus, blood, spleen, and LN, cells were stained with the following mAbs: anti-mouse CD45.2 FITC (clone 104), CD19 Brilliant Violet 510 (1D3), CD44 APC (IM7), CD62L PE (MEL-14) were purchased

from BD Biosciences. Anti-mouse CD4 eFluor®450 (RM4-5), CD8a Pe-Cyanine7 (53-6.7), CD90.2 (Thy-1.2) APC (53-2.1), and Foxp3 PE (FJK-16s) were purchased from eBioscience.

Cells were counted in Neubauer Chamber and approximately 500,000 cells were used for flow cytometry analysis. Briefly, cells were washed in DPBS and labeled with a cocktail of mAbs specific for cell surface Ag diluted in DPBS containing 2% FBS. After 20 min incubation at 4°C in the dark, labeled cells were washed in DPBS containing 2% FBS and resuspended in DPBS before analysis. For Foxp3 intracellular staining, cells were labeled for surface Ag, washed in DPBS, fixed, and permeabilized with fixation/permeabilization solution (Anti-Mouse/Rat Foxp3 Staining Set, eBioscience) according to the manufacturer's instructions and stained for intracellular Foxp3. Labeled cells were analyzed on a BD FACS Verse (BD Biosciences) using BD FACS Suite Software (BD Biosciences). Number of cells was calculated in function of the volume of cell suspension analyzed by the FACS Verse and multiplied by the dilution factor and the factor of proportion of cell suspension used for flow cytometry compare to the total volume of suspension.

TREC Quantification

PCR quantification (qPCR) of sjTREC and DJβTREC were performed according to a protocol adapted from Dulude et al. (18), using CD4 gene as a reference single-copy gene. Briefly, cells were lysed in lysing buffer containing Tris–HCl (10 mM; pH 8.3), Tween 20 (0.05%), Igepal (0.05%), and proteinase K (100 µg/ml) for 30 min at 56°C followed by proteinase K inactivation (10 min at 95°C). DNA from cell lysates was preamplified in an iCycler (Bio-Rad) using outer primers (**Table 1**) and GoTaq® Flexi DNA Polymerase (Promega) with the following conditions: initial denaturation at 95°C for 10 min; 22 cycles of amplification at 95°C for 30 s; 60° for 30 s; 72°C for 2 min; final elongation 72°C for 10 min; and cooling at 15°C. In this first-step of amplification, CD4 gene was coamplified together with the sj- or DJβTREC. Plasmids containing CD4 and sj61 or DJβ4 sequences were preamplified in the same way and used to generate standard curves. PCR products were diluted and CD4 and TREC amplicons were quantified by qPCR in a LightCycler480 thermocycler (Roche Diagnostics) using Takyon™ No Rox SYBR MasterMix Blue dTTP (Eurogentec) and inner primers (**Table 1**) with the following conditions: 5 min of initial denaturation at 95°C; 40 cycles of amplification at 95°C for 10 s; 60°C for 15 s; 72°C for 10 s; and cooling at 40°C. Results were analyzed on the LightCycler480 Software and expressed in number of TREC per 10^6 cells. All probes were purchased from Eurogentec. Total sjTREC content was estimated by multiplex quantification of δREC1/jα61 and δREC1/jα58 rearrangements, since they account for almost 100% of total sjTREC frequency (18). Similarly, dβTREC content was obtained by multiplex nested-PCR of dβ1 rearrangement with Jβ1.1-1.6. An internal control was added to each run of qPCR to evaluate the run-to-run variation. When the SD for the control sample was above 10%, a correction factor (TREC content of control sample in this run/mean TREC content of control sample for all run) was applied to each sample of the run.

TABLE 1 | Outer and inner primers for T-cell receptor excision circle quantification.

Name	Sequences out	Sequences in
CD4 1	CCAACCAACAAGAGCTCAAGGA	AGCTCAAGGAGACCACCATGT
CD4 2	CCCAGAATCTTCCTCTGGT	TGGTCAGAGAACTTCCAGGT
Jα61	AACTGCCTGGTGTGATAAGAT	GGAGTATCTCTTTGGAGTGA
Jα58	CCCAGGACACCTAAAAGGAT	AACTCGCACAGTGGAGGAAA
REC1	AGTGTGTCCTCAGCCTTGAT	GAAAACCTCCCCTAGGAAGA
dβ1	TATCCACTGATGGTGGTCTGTT	GACGTTGGCAGAAGAGGATT
Jβ1.1	CATGTTTGACATTGCCACAAGT	AGCGATTACTCCTCCTATGGT
Jβ1.2	CTCTCTTCACCCCTTAAGATT	GTAAAGGAACCAGACTCACAGTT
Jβ1.3	TGAGGCTGGATCCACAAAGGT	TCAAGATGAACCTCGGGTGGA
Jβ1.4	GGGCCATTAGGAAACGTGAT	GCAGGAAGCATGAGGAAGTT
Jβ1.5	GGAGGAAGGAAGGATGGTGA	CAGAGTCCTGCCTCAAAGAA
Jβ1.6	CCTGTGACATGCCTCATGGTA	TCAGGTCTCAGGGATCTAAGA

GH Supplementation

Mice were daily injected i.p. with either human recombinant GH (1 mg/kg in 100 µl DPBS, Genotonorm, Pfizer) or DPBS as control for 6 weeks. Two weeks before injection, a blood sample was taken from the tail for TREC quantification. At day 0 before injection and once per week after the beginning of the treatment, glycemia and weight were measured to follow the effect of GH treatment and a blood sample (130 µl in WT and 65 µl in KO mice) was taken from the tail weekly for flow cytometry analysis or TREC quantification (each analysis was alternatively performed every second week). After 6 weeks of treatment, mice were euthanized by i.p. injection of ketamine (100 mg/kg)–xylazine (10 mg/kg) followed by cardiac puncture and thymus, spleen, LN, and liver removal.

Igf1 Quantification by Real-Time Quantitative PCR

Igf1 transcripts were analyzed as previously described (19). RNA extraction was performed using NucleoSpin® RNA kit (Macherey-Nagel) according to the manufacturer's instructions. Liver tissue extracts kept at 4°C in RNAlater (Qiagen) and thymic cell suspensions were disrupted in lysis buffer containing β-mercaptoethanol and stored at −80°C until RNA extraction. After extraction, RNA concentration was measured by NanoDrop ND-1000 (Thermo Scientific) and 500 ng were used for reverse-transcription with oligo-dT using Transcriptor first strand cDNA synthesis Kit (Roche). Quantitative PCR was performed in the iCycler (Bio-Rad) using Taqman probes technology and iQSupermix (Bio-Rad) with the following primers: *Igf1* forward CAGGCTATGGCTCCAGCATT; *Igf1* reverse ATAGAGCGGGCTGCTTTTG; probe 6-FAM-AGGGC ACCTCAGACAGGCATTGTGG-BHQ-1. Mouse hypoxanthine–guanine phosphoribosyltransferase (HPRT, Mm01324427_m1 TaqMan Gene Expression Assays, Applied Biosystems) in the following conditions: polymerase activation at 50°C for 2 min; preliminary denaturation at 95°C for 10 min; 50 cycles of amplification 95°C for 15 s; 60°C for 1 min. Mouse *Hprt* was used as a housekeeping gene. Number of copies for *Hprt* and total *Igf1* were calculated from the linear regression of standard curve generated from serial dilution of plasmids specifics for each gene.

Dexamethasone (DXM) Administration

Mice were injected i.p. with 20 mg/kg Dexamethasone dihydrogenophosphat-Dinatrium (Aacidexam 5 mg/ml, Aspen) or DPBS as control. The day of injection was referred to as d0. MRI sessions were performed at day 0, 2, 5, 10, and 14 to follow thymic involution and recovery. At d15, mice were euthanized and thymus, blood, spleen, and LN were removed for further analysis.

MRI Data Acquisition and Processing

Anesthesia was induced with isoflurane 4% in air, and then maintained by reducing the ratio to 1.5% for the duration of the acquisition (flow rate: 0.8 l/min). The mice were placed prone in a stereotaxic holder (Minerve, France). The breathing rate was monitored during the entire scan and the body temperature maintained at 37 ± 0.5°C with an air warming system (Minerve, France). MRI anatomical images were acquired on a 9.4 T MRI DirectDrive VNMRS horizontal bore system with a shielded gradient system (Agilent Technologies, Palo Alto, CA, USA) and a 40-mm inner diameter volumetric coil (Agilent Technologies, Palo Alto, CA, USA). Fast spin echo multislices sequence were acquired using the following parameters adapted from Brooks et al. (20) and Beckmann et al. (21): $TR/TE_{eff} = 2{,}000/40$ ms, matrix = 192 × 192, FOV = 20 mm × 25 mm, 10 contiguous slices focused on the region of interest (thickness = 1.0 mm, in-plane voxel size: 0.104 mm × 0.130 mm). Anatomical images were analyzed using PMOD software version 3.6 (PMOD Technologies Ltd., Zurich, Switzerland). The thymus was manually segmented, thanks to its difference in signal intensity from the surrounding tissues, on each contiguous slice (thereafter refer as region-of-interest, ROI). The PMOD tools allow direct computing of the organ volume, by multiplying the effective slice thickness with the surface areas of each ROI.

Statistical Analysis

Statistical analyses were performed on the Prism 4.0 software (GraphPad). Kolmogorov–Smirnov and Shapiro–Wilk normality tests were performed to evaluate the Gaussian distribution of results. Unpaired *t*-test was applied when Gaussian distribution was verified, and Mann–Whitney test for non-Gaussian distributions. For multi-parametric analysis of GH supplementation, two-way ANOVA with Bonferroni post-test was used.

RESULTS

Somatotrope Deficiency in GhrhKO Mice Affects Lymphoid Organ Weight and Cellularity

As previously described (14), *Ghrh*KO mice have a dwarf phenotype, with an adult weight 50% smaller than control counterpart (**Table 2** and Table S1). Female but not male mutant mice catch up weight of WT mice with age, but it is mainly due to fat accumulation (personal observation). Both spleen and thymus are smaller in *Ghrh*KO mice (**Table 2** and Table S1). When corrected to total body weight, the spleen remains proportionally smaller in mutant mice (about 40% reduction). On the opposite, the relative thymus weight is similar between normal and mutant mice and decreases at 18 months of age in both strains (**Table 2** and Table S1). Since the absolute weight in *Ghrh*KO thymus is stable with time, unlike WT mice, this decrease in relative weight could not be attributed to thymic atrophy, but rather to the significant increase in total body weight.

The absolute number of $CD45^+$ leukocytes in spleen and LNs and $CD90.2^+$ (Thy1.2) thymocytes in thymus are also reduced in *Ghrh*KO mice compared to WT mice (**Table 2** and Table S1). When corrected to the weight of the corresponding organ, they are no differences in relative cellularity between both strains (**Table 2** and Table S1). Both mutant and normal mice undergo a loss of cell number and density with age in the two organs. Moreover, thymic involution is clearly evidenced by the age-dependent loss of the absolute thymocyte number in both strains. Loss of cells is accompanied by reduction in tissue weight in 18-month-old

TABLE 2 | Effects of somatotrope deficiency on weight and number of cells of lymphoid organs.

	3 Months		6 Months		18 Months	
	C57BL/6 wild-type (WT) (n = 3♂ 12♀)^c	***Ghrh*KO (n = 10♂ 6♀)^c**	**C57BL/6 WT (n = 2♂ 6♀)^b**	***Ghrh*KO (n = 4♂ 12♀)^c**	**C57BL/6 WT (n = 3♂ 6♀)^c**	***Ghrh*KO (n = 1♂ 5♀)^a**
Body weight (g)						
Male ♂	24.5 ± 0.18	13.1 ± 0.18***	32.0 ± 1.15$^{(a)}$	16.7 ± 0.41***$^{(a)}$	33.6 ± 5.01	15.9
Female ♀	21.3 ± 0.36	11.8 ± 0.14***	24.4 ± 0.75$^{(a)}$	15.9 ± 0.54***$^{(a)}$	27.0 ± 0.23$^{(a,b)}$	24.7 ± 2.26$^{(a,b)}$
Thymus						
Absolute weight (mg)	53.2 ± 4.28	29.6 ± 2.56***	58.6 ± 5.61	29.62 ± 1.87***	23.4 ± 2.78$^{(a,b)}$	29.9 ± 2.40
Relative weight (mg/g of body weight)	2.5 ± 0.24	2.2 ± 0.25	2.3 ± 0.26	1.7 ± 0.12	0.9 ± 0.12$^{(a,b)}$	1.2 ± 0.12$^{(a,b)}$
Absolute number of cells (×10^6)	47.3 ± 6.11	32.4 ± 4.56*	35.5 ± 3.14	20.8 ± 1.82*$^{(a)}$	16.3 ± 7.90$^{(a)}$	ND
Relative number of cells (×10^4/mg of thymus)	84.3 ± 15.76	107.9 ± 18.00	62.7 ± 5.58	76.3 ± 7.93	38.7 ± 12.27$^{(b)}$	ND
Spleen						
Absolute weight (mg)	79.1 ± 3.19	31.0 ± 2.04***	77.7 ± 9.4	35.6 ± 1.86***	97.4 ± 4.51$^{(a)}$	59.2 ± 8.19***$^{(a,b)}$
Relative weight (mg/g of body weight)	3.6 ± 0.15	2.2 ± 0.06***	3.1 ± 0.43	2.2 ± 0.10**	3.4 ± 0.25	2.2 ± 0.19**
Absolute number of cells (×10^6)	44.8 ± 4.61	20.3 ± 2.05***	51.3 ± 6.08$^{(a)}$	18.1 ± 1.78***	21.5 ± 3.18$^{(a,b)}$	18.5 ± 3.68
Relative number of cells (×10^4/mg of spleen)	56.7 ± 5.11	63.6 ± 3.53	52.5 ± 3.79	50.2 ± 4.25$^{(a)}$	21.5 ± 2.22$^{(a,b)}$	34.8 ± 7.36$^{(a)}$

Data (mean ± SEM) are representative of one^a, two^b, or three^c independent experiments.

ND, not determined.

*Unpaired t-test or Mann–Whitney test were used according to the Gaussian distribution of each set of data. Significant difference from age-matched WT mice ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$; significant difference compared to 3-$^{(a)}$ or 6-$^{(b)}$month-old mice from the same strain.*

C57BL/6 mice. On the contrary, thymic T-cell disappearance in *Ghrh*KO mice seems to be equally compensated by fat or other tissue as thymus absolute weight does not vary with time.

Somatotrope Deficiency Is Associated With Minor Changes in Thymocytes Subsets Repartition

Thymopoiesis is first assessed by analyzing thymocyte phenotype. Thymic T-cells are subdivided in four subpopulations based on their expression of CD4 and CD8 surface molecules. The most immature CD4$^-$CD8$^-$ subset is called double-negative (DN) cells. They evolve and acquire expression of both CD4 and CD8 to become double-positive (DP) cells, then mature to single-positive (SP) CD4$^+$ or CD8$^+$ cells by losing expression of either CD8 or CD4 molecules, respectively (**Figures 1A,B**; Table S2 in Supplementary Material). Flow cytometry analysis of thymic T-cell subpopulations shows a significant decrease (about one-third) in frequency of DN subset in *Ghrh*KO compared with C57BL/6 mice (WT: 3.3 ± 0.13% vs KO: 2.2 ± 0.12% at 3 months and WT: 3.8 ± 0.31% vs KO: 2.5 ± 0.07% at 6 months), compensated by an increase in DP cells at 3 months (84.8 ± 0.70% for WT vs 87.0 ± 0.36% for KO) and CD8$^+$ SP cells at 6 months (2.1 ± 0.11% for WT vs 2.8 ± 0.12% for KO;

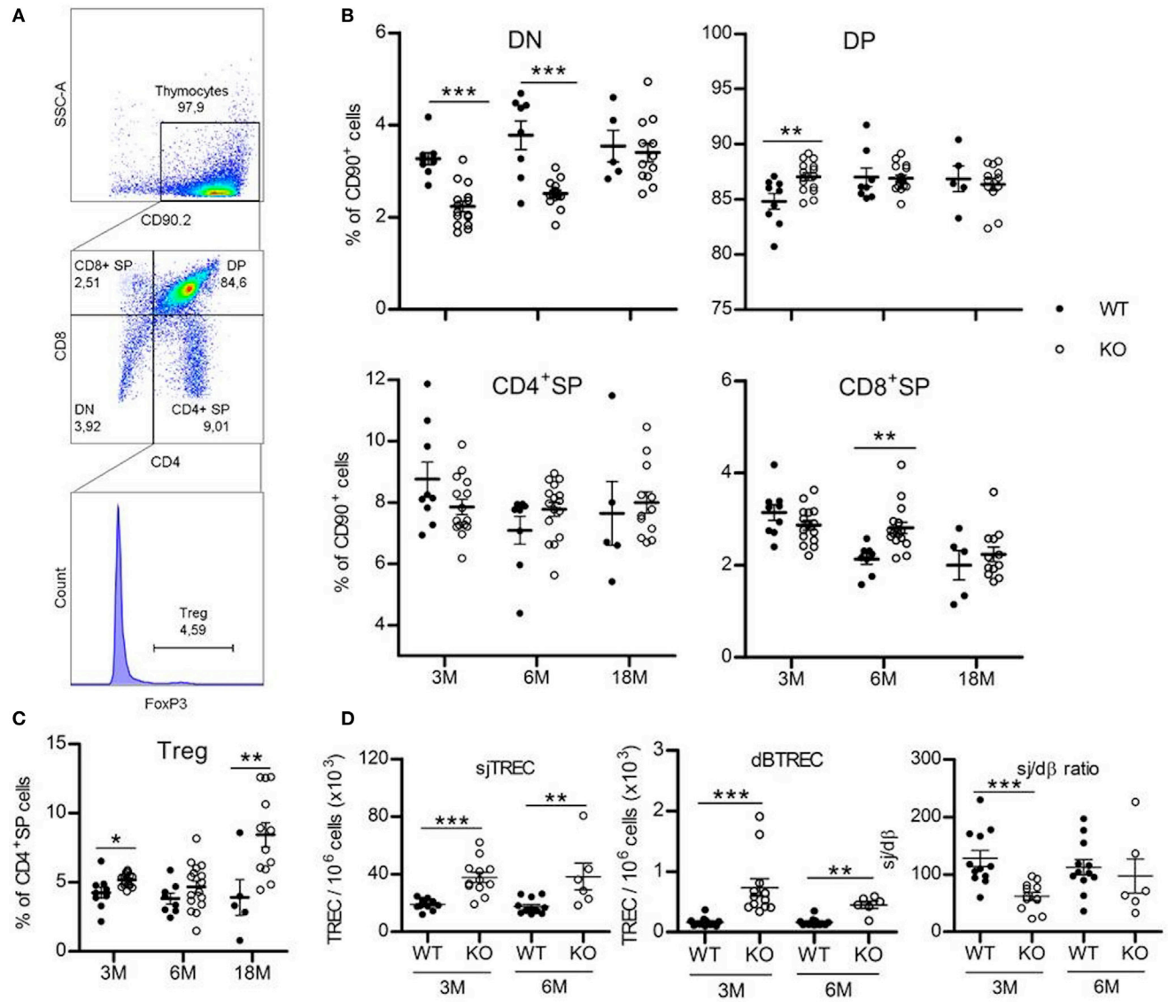

FIGURE 1 | Thymus phenotype and function. **(A)** Representative flow cytometry plots showing the four thymic subpopulations double-negative (DN) (CD4$^-$CD8$^-$), double-positive (DP) (CD4$^+$CD8$^+$), CD4$^+$ single-positive (SP) (CD4$^+$CD8$^-$), and CD8$^+$ SP (CD4$^-$CD8$^+$) cells, identified within the CD90.2$^+$ population. FoxP3$^+$ Treg cells are studied inside the CD4$^+$ SP population. 30,000 events are recorded. **(B,C)** Frequencies of the four thymic T-cell subpopulations **(B)** and FoxP3$^+$ Treg cells **(C)** in thymus of KO (white circles, n = 12–16) and wild-type (WT) (black circles, n = 5–9) mice at 3, 6, or 18 months. Data (mean ± SEM) are representative of two to three independent experiments. Unpaired t-test was used for statistical analysis. **(D)** sjTREC, dβTREC, and sj/dβ ratio in splenocytes of KO (white circles, n = 12) and WT (black circles, n = 6–12) mice at 3 and 6 months are shown. Data (mean ± SEM) are representative of two independent experiments. Unpaired t-test was used for statistical analysis. $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$.

Figure 1B; Table S2 in Supplementary Material). At 18-month-old, frequency of DN cells in mutant mice increases compare to younger animals, to be equal to values in normal mice (WT: 3.4 ± 0.20% and KO: 3.55 ± 0.34%). In addition, Treg cells in CD4$^+$ SP subset (**Figure 1C**; Table S2 in Supplementary Material) increases in thymus of mutant mice ($p = 0.011$ for 3 months, $p = 0.208$ for 6 months, and $p = 0.013$ for 18 months).

Taken together, those results could indicate a faster commitment of *Ghrh*KO DN cells in the thymopoietic process, leading to lower frequency of the most immature thymic T-cells and increasing number of the subsequent stages of development. Finally, at advanced age (18 months), thymocyte frequency and number return to values equivalent to WT mice.

TREC Numbers Are Increased in GhrhKO Mice

A widespread method to assess thymic function is the quantification of TREC, the small circles of DNA produced during TCR rearrangement of segment genes coding for α and β chains of the T-cell receptor for the antigen (TCR). They offer the advantage to be stable in cells and as duplicated during mitosis, slowly diluted by cell proliferation. dβTREC are created at an early stage of thymopoiesis during β chain rearrangement in DN cells and sjTREC are products of δ locus deletion in DP and SP cells. Therefore, sjTREC are present in almost all recent thymic emigrants (RTE) and are markers of thymic output, while the ratio of the later one (sj) by the earliest one (dβ) reflects intrathymic proliferation of thymocytes (18). Measurement of TREC content in splenocytes of *Ghrh*KO mice reveals a twofold increase number of sjTREC compared with age-matched WT mice and a threefold and fourfold increase in dβTREC content at 3 and 6 months, respectively (**Figure 1D**). Conversely, the intrathymic proliferation estimated by the sj/dβ ratio is reduced in 3-month-old mutant mice. Results were similar in blood PBMC (Figure S1 in Supplementary Material). Collectively, those results indicate an increased thymic output of naïve T cells with decreased intrathymic proliferation. Moreover, TREC analysis shows that thymopoiesis is not impaired at 6 months, with values similar to 3-month-old mice in both WT and mutant (**Figure 1D**). Unfortunately, we were unable to measure TREC in 18-month-old mice due to the difficulty to obtain such old animals. However, data could be inferred from GH supplementation experiment, where TREC are measured in blood of 3- and 18-month-old mice 2 weeks before GH treatment and show a clear reduction of thymic output at 18 months in both strains while intrathymic proliferation is reduced only in WT mice (**Figure 3E**; Figure S1 in Supplementary Material). This reveals that a decline in thymopoiesis occurs similarly in WT C57BL/6 and *Ghrh*KO between 6 and 18 months of age.

Lymphocytes Distribution in Periphery Is Slightly Disturbed in GhrhKO Mice

To determine if somatotrope deficiency could also affect peripheral lymphocytes, flow cytometry analyses of spleen, inguinal LNs, and blood were performed. *Ghrh*KO mice present an approximately 10% reduction of B-cell frequency at 3 and 6 months, while T-cell proportion is increased (**Figure 2A,B**; **Table 3**). Differences tend to attenuate with aging, since 18-month-old KO mice are not different from age-matched WT mice. Among T cells, distribution of CD4 and CD8 T cells is also disturbed in mutant mice, but depends on age and organ analyzed. Indeed, there is no difference in the spleen of 3-month-old mutant mice. At 6 months, the slightly decreased proportion of CD4 T cells and the increased proportion of CD8 T cells in *Ghrh*KO observed when comparing by *t*-test is not significant when two-way ANOVA was used (**Table 3**; Table S3 in Supplementary Material) suggesting the time variation biased the difference between mutant and WT mice. On the contrary, the increased proportion of CD4 and the decrease in CD8 is observed at 18 months whatever the test used (**Figure 2B**; **Table 3**; Table S3 in Supplementary Material). In LN, mutant mice shows a constant increased in CD4 T cells and decreased in CD8 T-cell frequencies (about 5%) compared with normal mice (**Table 3**; Table S3 in Supplementary Material). The CD4/CD8 ratio in LN and blood of both normal and mutant mice decreases with time, an expected effect of aging (22).

Differences in lymphocytes subsets distribution were further studied by exploring the naive or memory character of CD4 and CD8 T cells. In mice, the low expression of CD44 is specific to naive T cell, while differential expression of L-selectin CD62L allows to separate central memory T cells (TCM) and effector memory T cells (TEM) in the CD44^{high} quadrant. The pool of naive T cells (CD44^{low}CD62L^{hi}) is greater in *Ghrh*KO compared with WT mice, with an doubled frequency of naive cells inside the CD4 subset and a moderate increase of 10% among CD8 T cells (**Figure 2C**; **Table 3**). On the contrary, the proportion of memory cells is reduced, mostly through the CD44^{hi}CD62L^{hi} central memory (TCM) pool. This is consistent with the observed increase of TREC in *Ghrh*KO mice, since TREC are present in newly formed naive cells exported from the thymus.

Finally, since Treg proportion seems higher in thymus of *Ghrh*KO mice, FoxP3$^+$ Treg cells were next analyzed among CD4 T cells in periphery. As expected, Treg compartment enlarges with time, but in similar proportions between *Ghrh*KO and WT mice, except in the spleen of 3-month-old *Ghrh*KO mice where Treg proportion is higher than in control mice ($p < 0.001$; **Table 3**; Table S3 in Supplementary Material). Taken together, those data demonstrated changes in the distribution of some lymphocytes subsets in peripheral lymphoid organs, without any evidence of lymphopenia.

GH Supplementation Is Unable to Restore Normal Phenotype in GhrhKO Mice

To determine if GH supplementation could restore a normal phenotype in *Ghrh*KO mice, animals were injected with a replacement dose of human recombinant GH or DPBS as control. Young and old mutant and WT mice were tested to see if GH action varies with aging. The efficiency of GH treatment was validated by its effects on weight and liver IGF1 stimulation (**Figures 3A,C**). Indeed, both *Ghrh*KO and WT mice show significant weight gain during GH treatment, compared with control mice, even though the effect is far more important (Bonferroni test following two-way ANOVA: $p < 0.001$ KO vs WT for 3 months mice after 6 weeks of GH treatment) in KO mice with a ~45% increase for 3 months

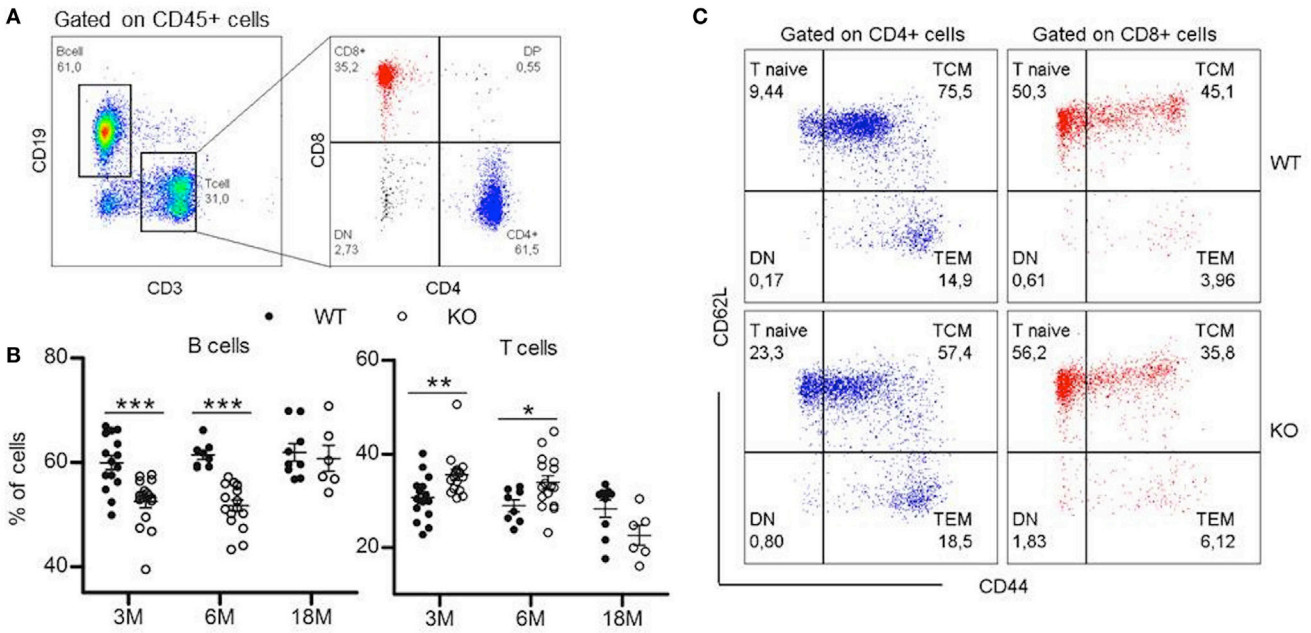

FIGURE 2 | Lymphocytes distribution in periphery. **(A)** Representative flow cytometry plots showing immunophenotyping of peripheral lymphoid compartments (blood, spleen, and lymph nodes). CD19$^+$ B cells and CD3$^+$ T cells are analyzed within the CD45$^+$ population. T cell population is divided into CD4$^+$ and CD8$^+$ T cells. 20,000 CD45$^+$ events are recorded. **(B)** Proportions of B and T cells in the spleen of KO (white circles, $n = 6$–16) compared with wild-type (WT) (black circles, $n = 8$–15) mice at 3, 6, and 18 months. Data (mean ± SEM) are representative of two to three independent experiments except for the KO18M group analyzed in a single experiment. Unpaired *t*-test was used for statistical analysis. $^{***}p < 0.001$, $^{*}p < 0.05$. **(C)** Representative flow cytometry plots for phenotyping of naïve and memory T cells in the spleen of 3-month-old WT and KO mice. Naïve (CD44^{low}CD62L^{hi}), TCM (CD44^{hi}CD62L^{hi}), and TEM (CD44^{hi}CD62^{low}) cells are analyzed within the CD3$^+$CD4$^+$ (blue) or CD3$^+$CD8$^+$ (red) populations.

compared with ~11 for WT mice (**Figure 3A**). Spleen and thymus in mutant mice are also significantly heavier in GH-treated relatively to DPBS-injected, particularly the spleen whose mass is doubled compared with control-injected mice (**Figure 3B**). Moreover, GH treatment stimulates *Igf1* expression in the liver of *Ghrh*KO mice, although it does not reach values in normal mice (**Figure 3C**). This demonstrates that a 6-week treatment with GH is able to compensate the somatotrope deficiency by inducing weight gain and IGF-1 stimulation without completely restoring values measured in normal mice.

The impact of GH treatment upon immune system was first evaluated in the thymus. No differences are observed between GH- or control-injected mice regarding the distribution of the four thymocyte sub-population and Treg cells in the thymus (**Figure 3D**). Similarly, TREC number and Sj/dβ ratio during GH treatment does not significantly differ from control-injected mice (**Figure 3E**). Young animals exhibit a decrease in both Sj and dβTREC during treatment with GH or vehicle alone that does not appear in old mice. Nevertheless, no differences appear at 3 or 18 months neither between control and GH-injected animals. This suggests that aging is not a factor that sensitizes mice to GH.

Next, the ability of GH injections to restore lymphocytes phenotype in periphery was also studied. There were no detectable differences in the frequency of all lymphocytes subtypes in the LN of GH-treated mutant mice compared with control (Figure S2 in Supplementary Material, lower panel). However, in the spleen, GH significantly increased B-cell frequency and decreased T cells ($p = 0.002$ and 0.001, respectively; Figure S2 in Supplementary Material, upper panel), increased frequency of CD4 T cells ($p = 0.043$), as well as increased CD8 naive pool and decreased CD8 TCM ($p = 0.025$ and 0.005, respectively, data not shown). The other subtypes were not affected by GH treatment. Finally, a blood sample was taken 1 week of two to follow the frequency of lymphocytes across the time. Flow cytometry experiments revealed huge week-to-week variations in all the groups studied (Figure S2 in Supplementary Material). Even if two-way ANOVA analysis revealed some significant effects of the treatment (decreased B cells, increased T cells, and decreased CD8 TCM in KO3M; increased CD4 TEM and decreased CD8 naïve T cells in KO18M, data not shown), it is still unclear if the differences are due to a real effect of GH or to random variations that would affect both control and GH-treated mice. Globally, those results suggest that GH supplementation is not sufficient to restore a normal phenotype in *Ghrh*KO mice.

GhrhKO Mice Show a Delayed Recovery of Their Thymic Volume After DXM-Induced Thymic Atrophy

Stress hypothesis suggests that somatotrope deficiency could lead to inability for the immune system to correctly respond to stressful events. To explore this hypothesis, *Ghrh*KO mice were challenged with DXM, a synthetic GC that induces a reversible thymic atrophy. After a single DXM injection, MRI sessions were

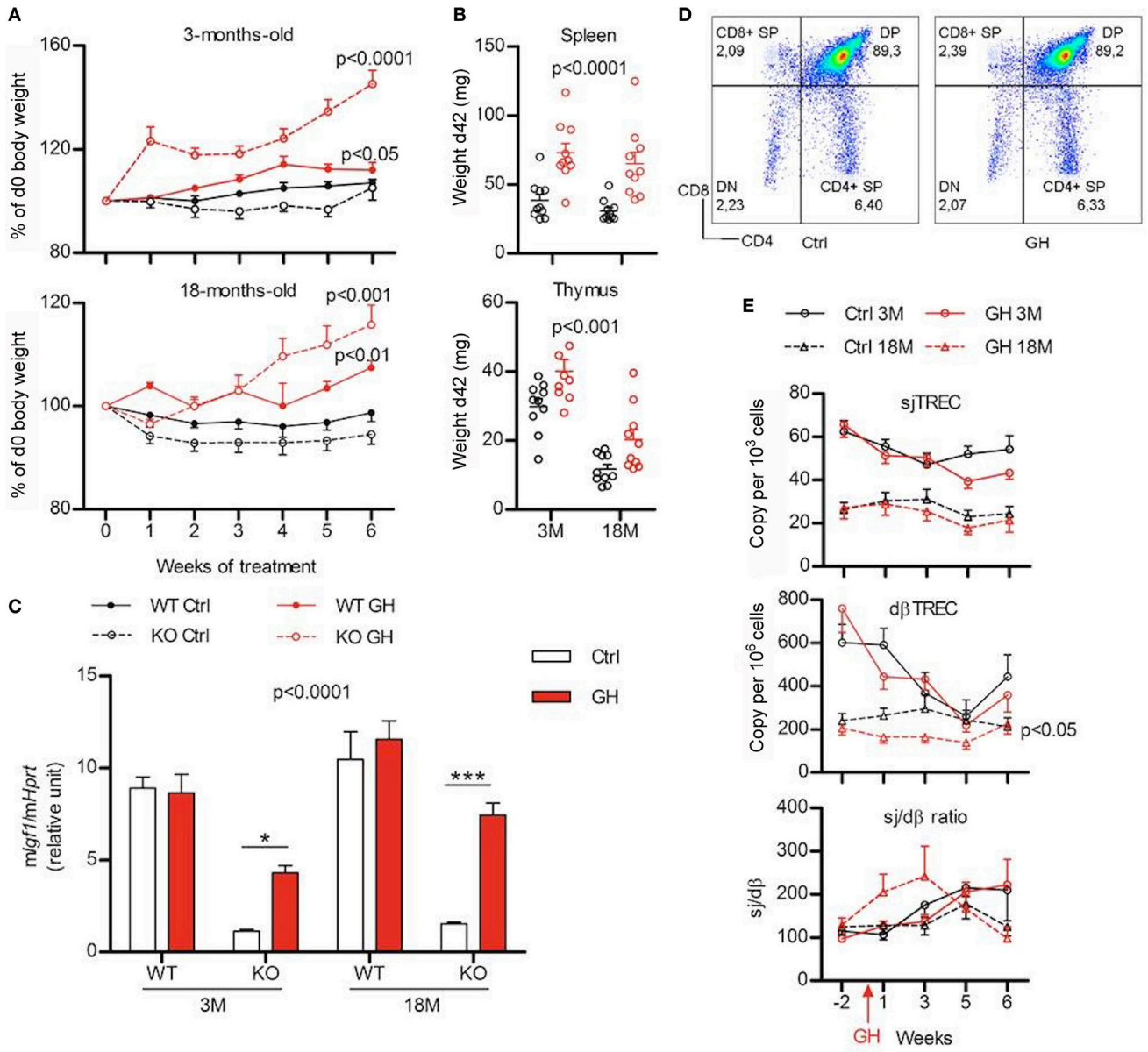

FIGURE 3 | Effects of growth hormone (GH) supplementation (6 weeks) on metabolic and immune parameters. **(A)** Weight variations expressed in percentage of starting value (weight at d0) are shown for 3- (above) and 18-month (below) old wild-type (WT) (filled line) and KO mice (dotted line) injected with GH (red) or control Dulbecco's phosphate-buffered saline (black). Two-way ANOVA test (time and treatment) was used for statistical analyses (n = 7–10 per group). **(B)** Igf1 expression in the liver of KO and WT mice after 6 weeks of GH (red) or control (white) treatment was measured by real-time quantitative PCR and normalized to expression of Hprt. Two-way ANOVA test (treatment and strain) with Bonferroni post-test were used for statistical analyses (n = 6–10 per group). ***$p < 0.001$, *$p < 0.05$. **(C)** Absolute weight of spleen (above) and thymus (below) of young and old KO mice after 6 weeks of GH (red) or control (black) treatment. Two-way ANOVA test (treatment and age) was used for statistical analyses (n = 10 per group). **(D)** Representative flow cytometry plots of immunophenotyping of thymus from control- and GH-injected mouse. **(E)** Variations of peripheral sjTREC, dβTREC, and sj/dβ ratio during GH supplementation (red) and control (black) in 3- (filled line) and 18-month-old (dotted line) KO mice. Two-way ANOVA test (time and treatment) was used for statistical analyses (n = 10 per group). **(A,B,C,E)** Data (mean ± SEM) are representative of two independent experiments.

performed at days 0, 2, 5, 10, and 14 to quantify thymic volumes in a longitudinal follow-up (**Figure 4**). Both WT and KO mice showed a significant loss of more than 50% of thymic volume at day 2, demonstrating the DXM-induced atrophy. At day 5, thymic volume increased to reach normal values as soon as day 10 in WT mice and even rise above starting volume at day 14. Even if statistical significance was low (*Ghrh*KO d10 vs *Ghrh*KO d0: $p = 0.616$, *Ghrh*KO d14 vs *Ghrh*KO d0: $p = 0.505$, paired t-test), *Ghrh*KO DXM-injected mice did not seem to completely restore their thymic volumes at days 10 and 15 (only 70% of the starting volume) but volumes were not significantly different from control-injected group, probably because of high variation rate in

TABLE 3 | Effects of somatotrope deficiency on frequency of lymphocytes subpopulations in spleen, lymph node (LN), and blood.

Frequency (% of parent population)	3 Months		6 Months		18 Months	
	C57BL/6 wild-type (WT) (n = 15)[c]	*Ghrh*KO (n = 16)[c]	C57BL/6 WT (n = 8)[b]	*Ghrh*KO (n = 16)[c]	C57BL/6 WT (n = 9)[c]	*Ghrh*KO (n = 6)[a]
Spleen						
B cell	60.0 ± 1.36	52.5 ± 1.18***	61.4 ± 0.85	51.7 ± 1.06***	61.9 ± 1.67	60.8 ± 2.52
T cell	30.6 ± 1.23	35.5 ± 1.17**	28.9 ± 1.25	33.9 ± 1.41*	28.3 ± 1.84	22.6 ± 2.12*
T CD4	57.4 ± 0.66	56.2 ± 1.04	57.0 ± 0.40	54.3 ± 0.86*	55.7 ± 1.08	62.9 ± 2.52*
T CD8	36.7 ± 0.61	36.9 ± 1.08	36.0 ± 0.67	38.8 ± 0.81*	37.1 ± 0.93	31.4 ± 2.83*
CD4 naive	12.0 ± 0.62	29.7 ± 1.30***	12.1 ± 0.75	21.0 ± 1.36***	3.4 ± 0.36	9.6 ± 2.67
CD4 TCM	61.7 ± 2.20	45.6 ± 1.42***	52.1 ± 2.03	50.7 ± 1.39	21.2 ± 2.57	21.5 ± 4.04
CD4 TEM	24.6 ± 1.62	23.7 ± 1.47	35.5 ± 2.08	27.9 ± 1.99*	74.7 ± 2.60	68.5 ± 6.33
CD8 naive	47.9 ± 1.05	57.5 ± 1.26***	47.1 ± 1.74	59.2 ± 1.55***	17.9 ± 2.18	29.8 ± 6.02
CD8 TCM	40.8 ± 1.31	31.0 ± 1.02***	42.6 ± 1.57	31.2 ± 1.48***	59.4 ± 3.69	44.5 ± 2.15**
CD8 TEM	7.1 ± 0.69	7.3 ± 0.59	9.4 ± 0.57	7.6 ± 0.42*	22.4 ± 2.89	24.4 ± 4.17
Treg	14.0 ± 0.38	17.3 ± 0.60***	17.9 ± 0.69	18.0 ± 0.61	29.3 ± 1.98	29.2 ± 2.43
LN						
B cell	33.8 ± 1.85	26.5 ± 1.56**	39.8 ± 3.42	27.6 ± 1.94**	53.6 ± 2.50	48.1 ± 4.08
T cell	63.2 ± 1.82	70.1 ± 1.60**	56.5 ± 3.43	68.2 ± 2.27*	43.3 ± 2.66	42.3 ± 4.04
T CD4	53.1 ± 0.71	57.8 ± 1.13**	50.4 ± 0.48	55.5 ± 1.05**	42.0 ± 0.95	48.4 ± 1.99*
T CD8	43.5 ± 0.74	39.0 ± 1.15**	44.5 ± 0.38	40.8 ± 1.00*	49.8 ± 1.07	42.4 ± 2.46*
CD4 naive	15.2 ± 0.97	35.7 ± 1.72***	16.6 ± 0.66	31.4 ± 1.78***	10.6 ± 1.33	24.5 ± 3.37**
CD4 TCM	72.1 ± 1.80	48.7 ± 1.95***	63.0 ± 4.68	54.3 ± 1.70*	52.9 ± 2.39	37.6 ± 3.25**
CD4 TEM	11.5 ± 0.99	13.4 ± 1.29	18.7 ± 4.17	13.3 ± 0.88	36.2 ± 3.01	37.1 ± 5.15
CD8 naive	57.6 ± 1.19	66.4 ± 1.21***	59.7 ± 1.31	65.1 ± 1.51	39.5 ± 2.72	40.2 ± 4.64
CD8 TCM	37.3 ± 1.25	25.4 ± 0.84***	34.5 ± 1.44	28.0 ± 1.73	54.1 ± 2.21	47.5 ± 4.29
CD8 TEM	2.6 ± 0.21	2.8 ± 0.21	3.5 ± 0.27	3.0 ± 0.25	5.9 ± 0.81	9.2 ± 1.66
Treg	12.5 ± 0.53	12.5 ± 0.22	16.1 ± 0.51	16.3 ± 0.70	30.3 ± 2.13	30.7 ± 2.12
Blood						
B cell	46.1 ± 2.65	43.3 ± 2.55	54.2 ± 3.10	43.1 ± 5.39	75.7 ± 3.58	51.2 ± 5.26**
T cell	38.7 ± 1.64	30.4 ± 3.62*	29.8 ± 3.83	33.3 ± 6.31	17.2 ± 2.00	26.5 ± 3.78*
T CD4	54.9 ± 1.55	55.3 ± 0.96	52.2 ± 1.94	49.6 ± 3.53	35.5 ± 2.03	51.5 ± 3.38**
T CD8	42.5 ± 1.58	37.3 ± 1.43*	44.9 ± 2.09	46.1 ± 2.95	58.2 ± 2.53	44.5 ± 3.79*
CD4 naive	15.8 ± 0.88	27.1 ± 4.20**	19.3 ± 1.93	31.9 ± 2.87**	18.7 ± 6.06	30.6 ± 4.45
CD4 TCM	76.7 ± 1.72	36.6 ± 5.31***	67.3 ± 2.22	49.9 ± 4.06**	51.9 ± 5.02	40.9 ± 4.03
CD4 TEM	6.6 ± 0.90	27.8 ± 7.45***	12.1 ± 1.79	14.0 ± 2.83	34.9 ± 6.08	28.3 ± 8.25
CD8 naive	53.4 ± 2.86	31.7 ± 6.19**	48.6 ± 3.18	44.9 ± 4.03	32.5 ± 5.99	38.8 ± 6.74
CD8 TCM	42.6 ± 3.00	42.8 ± 1.87	40.5 ± 2.03	41.52 ± 2.39	49.0 ± 5.05	50.5 ± 4.66
CD8 TEM	2.6 ± 0.46	17.4 ± 5.08*	8.4 ± 1.81	8.34 ± 1.71	20.8 ± 6.55	10.5 ± 2.94
Treg	9.2 ± 0.98	8.0 ± 0.63	7.5 ± 0.90	7.8 ± 0.44	13.0 ± 0.92	11.2 ± 1.99

Data (mean ± SEM) are representative of one[a], two,[b] or three[c] independent experiments.

*Values significantly different from age-matched WT mice ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.*

CD19$^+$ B cells and CD3$^+$ T cells are analyzed within the CD45$^+$ population. T cell population is divided into CD4$^+$ and CD8$^+$ T cells. 20,000 CD45$^+$ events are recorded. Naïve (CD44^{low}CD62L^{hi}), TCM (CD44^{hi}CD62L^{hi}), and TEM (CD44^{hi}CD62^{low}) cells are analyzed within de CD3$^+$CD4$^+$ or CD3$^+$CD8$^+$ populations. FoxP3$^+$ Treg cells are studied inside the CD4$^+$ SP population.

control mice. However, the recovery (assessed in percentage of corresponding starting value for each mouse) was significantly different between WT and KO mice at days 10 and 14 (**Figure 4B**). This suggests a delayed restoration of thymic volumes after DXM-induced thymic atrophy in somatotrope-deficient mice compared with WT mice. However, this difference was not supported by analysis of thymus weight and cellularity obtained after sacrificing the mice at day 15 (**Figure 4C**). Indeed, they were no difference between control and DXM-injected animals in both WT and KO mice regarding those parameters, neither for TREC intrathymic content. Nevertheless, intrathymic proliferation reflected by sj/dβ ratio was higher in DXM-injected compared with control for WT but not mutant animals (**Figure 4C**). This revealed an increased thymic activity in WT mice 2 weeks after DXM injection, which was not observed in *Ghrh*KO mice. Altogether, MRI analyses of thymic volumes and sj/dβ ratio results suggested a delayed and less efficient thymic recovery in *Ghrh*KO mice after DXM-induced thymic atrophy while weight and cell number measures showed normal thymic restoration after 15 days. This apparent discrepancy might be attributed to the individual variation bias that cannot be avoided when animals are sacrificed at each time point for organ weighing.

DISCUSSION

Despite a large amount of literature about the effects of GH upon immune system, its implication in immune physiology is still unclear and controversial. Most of the previous works were done in mouse model with multiple pituitary deficiencies (GH, PRL, and thyrotropic hormones), making it difficult to identify

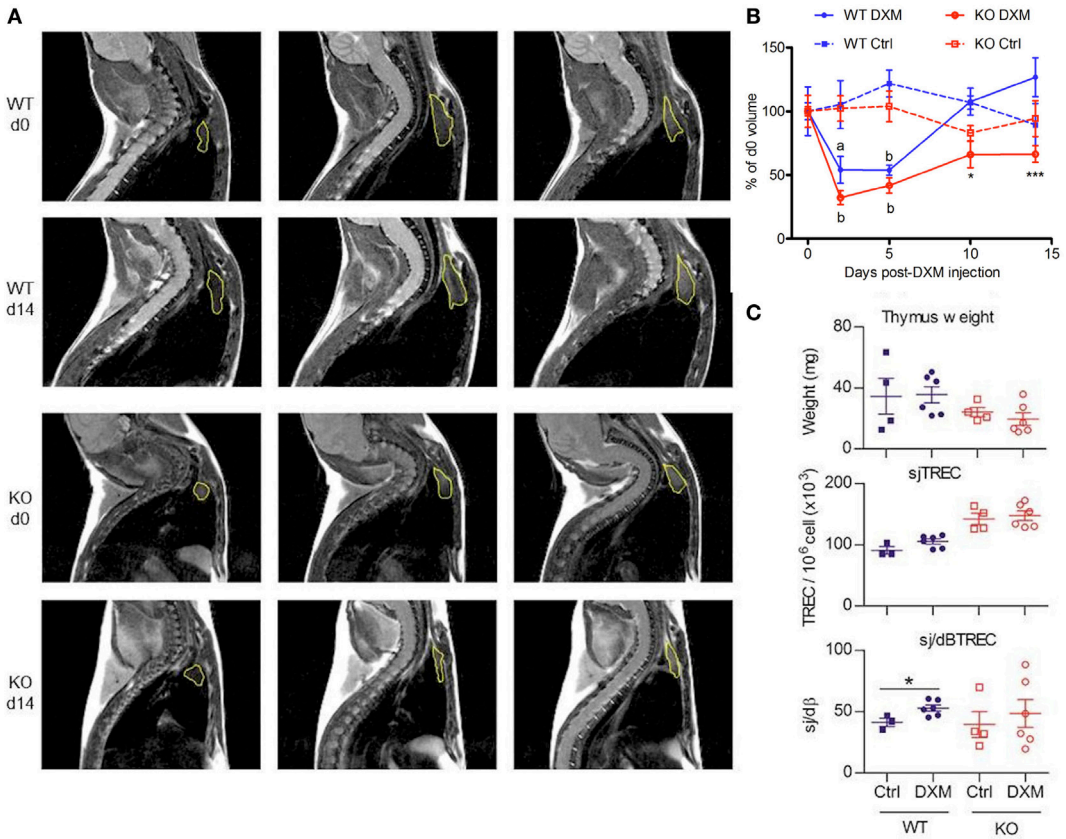

FIGURE 4 | Follow-up of dexamethasone (DXM)-induced atrophy and recovery. **(A)** Representatives MRI slices showing thymus [region-of-interest (ROI), yellow] of wild-type (WT) and KO at d0 and d14 after DXM injection. Lines show three successive slices around the maximal ROI. **(B)** Evolution of thymic volumes normalized in% of d0 value is shown for WT (blue) and KO (red) mice treated with DXM (filled line, $n = 6$ per group) or control solution (dotted line, $n = 4$ per group). Paired *t*-test was used for effect of DXM vs d0. ^{a}p: < 0.05, $^{b}p < 0.01$. Two-way ANOVA test (time and treatment) with Bonferroni post-test (WT vs KO for DXM or Ctrl treatment) was used for statistical analyze of genotype variation. $^{*}p < 0.05$, $^{***}p < 0.001$. **(C)** Thymus weight, sjTREC and sj/dβ ratio of WT (blue) and KO (red) mice at d15 post DXM- (circle, $n = 6$ per group) or control-injection (square, $n = 4$ per group). Unpaired *t*-test was used for statistical analysis. $^{*}p < 0.05$. **(B,C)** Data (mean ± SEM) are representative of two independent experiments.

the precise role of each hormone. Here, we investigated a model never used for immune characterization with a unique specific deficiency of the somatotropic GHRH–GH–IGF1 axis due to *Ghrh* deletion, in an attempt to elucidate the physiological role of somatotropic hormones in immune system development and function. We found no severe thymic or immunological defect in those mice. Within the thymus, they present a slight reduction in the proportion of the most immature thymocyte subset, but the relative weight and cellularity of this primary immune organ is similar to that in normal mice, even in old animal.

Age-induced changes in lymphocytes distribution in peripheral lymphoid organs were studied in mutant and *Ghrh*$^{-/-}$ mice. It is important to note that we cannot exclude some bias (1 of 20 parameters will reach a $p < 0.05$ randomly, cohort effects in aged animals, etc.) in analysis and interpretation of so many parameters. Therefore, careful and critical interpretation should be applied. Here, only consistent repeated results were taken into consideration. A first conclusion drawn from analysis of spleen, LNs and blood was that the differences observed between normal and *Ghrh*$^{-/-}$ mice at 3 months (i.e., lower B-cell and higher T-cell frequencies and higher proportion of naïve T cells and diminution of memory pool in KO vs WT mice) were maintained throughout life. Altogether, analysis did not reveal a strong differential effect of aging on peripheral lymphocytes between *Ghrh*$^{-/-}$ and normal mice. Both maintained relatively constant proportion of B and T cells and, as expected, they experienced a shift in the pool of naïve to memory T cells, within which mostly TEM were increased. Frequency of CD4 T cells decreased in the blood and LN of normal and mutant mice, but an inverted increase of CD8 frequency was observed only in WT organs. This resulted in a decreased CD4/CD8 ratio in the two compartments of the two types of aged mice, although the intensity of this decrease was more important in WT mice.

In periphery, *Ghrh*KO mice exhibit a decreased frequency of B cells, concomitant with a rise in proportion of T cells. The spleen is the only immune organ that remains smaller than in the WT counterpart when taking into account the body weight of the animal. The pool of naïve T cells is more important in somatotrope-deficient mice, a result also supported by the higher number of TREC in spleen and blood of *Ghrh*KO mice. A 6-week

GH supplementation is unable to restore those parameters to normal in our experimental model. Nevertheless, *Ghrh*KO mice do not present any obvious immunodeficiency or thymic atrophy. Taking together, those results indicate that the integrity of the somatotrope axis is not required for T cell immune system development in basal conditions.

Our results are in contradiction with previous work conducted in multi-deficient dwarf mouse model (Snell–Bagg and Ames) where were observed lymphopenia, decreased relative weight thymus, early thymic involution and reduced primary immune response compared to non-dwarf animals (2–4). This immunodeficiency is characterized by decreased number of thymic cells and dramatic decreased in proportion of DP thymocytes (23). Moreover, GH treatment could partially restore immune parameters (3, 23). Therefore, authors concluded that GH had significant effect on T cell development within the thymus. However, in most of the studies demonstrating a decreased thymic cellularity, the absolute number of cells was compared without normalizing to the smaller size of dwarf mice (23), and it cannot be so assumed that the smaller thymus size is due to a direct effect of GH upon the thymus or to spatial pressure linked to growth retardation. We and others (10, 11) demonstrated that even though a diminished absolute number of cells, GH-deficient mice had a normal thymic cellularity when corrected to their smaller size.

Other works are in agreement with our findings in *Ghrh*KO mice. The B cell frequency decrease is coherent with the previously observed impairment of bone marrow B cell production in dwarf mice model (11, 24). Very interestingly, this specific absolute and relative decrease in spleen size has been observed in human with a GHRH receptor mutation leading to dwarfism, establishing a link between our animal model and observation in human (25). A team studied dwarf Snell–Bagg mice and found no differences in lymphocytes distribution or function in thymus, while the spleen demonstrated a higher frequency of T lymphocytes and lower frequency of B lymphocytes compared with control (10). The number of splenic cells—but not thymic lymphocytes—was 50% of normal counterparts when corrected to total body weight, similar to what we found in *Ghrh*KO mice. Another group, using a panel of mouse strains affected with different pituitary hormone deficiencies, showed that primary B cell development defect was not dependent on hypophysial hormones, but was controlled by thyroid hormones. Nevertheless, GH could be involved in B cell reduction within secondary lymphoid organs (11). Indeed, thyroid axis-deficient mice exhibit a defect in bone marrow B lymphopoiesis and normal splenic B cells frequency, while the opposite was found in GH-deficient *lit/lit* mice. In both cases, thymus was unaffected by hormone deficiency. Our study in *Ghrh*KO mice confirms these observations, since we observed an almost normal thymus, but diminished B cells frequency in periphery (spleen, LNs, and blood). This reduction goes along with increasing proportion of T cells. Interestingly, the growing literature about effects of pituitary hormones upon B-lymphopoiesis strongly suggests a role for GH and IGF-1 as positive regulators of B cells (11, 26–29). It has been shown that GH receptor has a wider expression on B cells that on T cells (50% compared with 20%, respectively) (30), suggesting a higher sensitivity to GH for this lymphocyte subset. Moreover, there are evidences that IGF-1 is able to increase the amount of bone marrow B lineages cells and splenic B cells as well as accelerate B cell reconstitution after bone marrow transplantation (26, 31). Similarly, GH-transgenic mice exhibits higher number of total lymphocytes, an effect more important in B cells than T cells (28). Taking this into account, it is not surprising that *Ghrh*KO and *lit/lit* mice display B lymphopenia.

One surprising result is the marked increase in the number of TREC in *Ghrh*KO mice, with an opposite decrease of sj/DJβTREC ratio. Previous works from our lab and others are in favor of a positive role of somatotrope hormones upon TREC production. In GH-deficient patient, withdraw of GH treatment induced a drop in sjTREC frequency and sj/DJβTREC ratio, followed by recovery after GH resumption (9). Furthermore, HIV+ patient treated with GH showed increased TREC frequency in PBMC (8). In mice, IGF-1 administration resulted in significant increase in TREC number measured in thymus and periphery (32). Therefore, we expected that GH and IGF-1 deficiencies could lead to TREC diminution measured in blood. One hypothesis to explain our opposite result here is that GH absence affects peripheral proliferation of cells and/or cell activation in response to antigen more than thymic proliferation. Indeed, TREC, which are excision circles of DNA resulting from T cell receptor rearrangement, are stable in the cell but not duplicated during mitosis, leading to their progressive dilution across peripheral proliferation. So, interpreting sjTREC content as a marker of thymic output should be done carefully, regarding this dilution bias. On the contrary, the intrathymic proliferation rate estimated by sj/dβTREC ratio is independent of peripheral proliferation since it represents the ratio between a TREC created lately in the thymus (sj) to one formed early (dβ). Here, the apparent increase in thymopoiesis indicating by the higher number of TREC could be a false interpretation due to a less important proliferation rate in periphery of *Ghrh*KO mice. *In vitro* and *in vivo* studies demonstrated that GH and IGF-1 are able to stimulate T cell proliferation (32, 33). Importantly, sj/dβTREC ratio could truly reflect a decreased intrathymic proliferation in young mutant mice, as expected according to studies described above. A second hypothesis would involve a decreased cell activation and is reinforced by the high frequency of naïve T cells found in somatotrope-deficient mice. If *Ghrh*KO lymphocytes are less sensitive to antigen stimulation, they do not undergo the activation process, which implicates clonal proliferation and induction of memory cells. Therefore, TREC are less diluted, and pool of naïve cells stays more important than in normal mice. This theory of hyposensibility to antigen activation could explain the decreased sensitivity of *Ghrh*KO mice to induction of EAE (34). Moreover, this is in agreement with the current stress hypothesis (13), according to which pituitary hormones are immunoregulators that counteracts negative effects of stress, including physiological and biological stress, like antigen challenge. The absence of one or more of those stress hormones could result in inability of the immune system to deal with stressful situations.

The stress hypothesis was first proposed by Dorshkind and Horseman based on their observation that mice deficient for GH/IGF-1, PRL, or thyroid hormones have a normal humoral

and cellular response (35), a result in contradiction with previous statements in literature (2, 4, 23, 36, 37). Afterward, they discovered that *Snell–Bagg* dwarf mice housed in non-stressful conditions, separately from their normal littermates, had no thymopoiesis defects, in contrast to animals held in less stringent conditions (13, 38). Reviewing literature in this context reconciles the contradictory findings about immunodeficiency in pituitary-deficient mice. Most of the studies showing a depressed immune system dependent on pituitary hormones (2–4) were conducted 40 years ago when housing conditions were less healthy and could be source of physiological and psychological stress. On the opposite, we and others (10, 38), keeping the animals in stress-limited and highly sanitary environment, found normal thymus and immune system. This hypothesis is reinforced by the evidence that GH can inhibit cortisol-induced lymphopenia in hypophysectomized rats (39). Moreover, GH secretion is stimulated after stress exposition (40). A mechanism for this GH inhibition of GCs action involves the Jak2/Stat5 pathway, one of the GH-signaling pathways (41). It has been shown that Stat5 protein can form a complex with the GC receptor which diminishes the activation of promoters containing GC response elements and therefore inhibits GC-induced gene activation (42). PRL, another pituitary hormone which share the Stat5 signaling pathway, has been shown to suppress *in vivo* lymphocytes apoptosis induced by DXM, a synthetic GC (43). The role of PRL as anti-stress hormone was confirmed in mouse experimental *Trypanosoma cruzi* infection, characterized by increased levels of GC, and inversely decreased levels of PRL, and where PRL restoration limited thymic atrophy and DP thymocytes apoptosis (44). It is plausible that GH, sharing the transduction pathway with PRL, could act through a similar mechanism on stress-induced immunosuppression. The presently described *Ghrh*KO mouse constitutes an interesting experimental model to assess this question. According to the stress hypothesis, somatotrope deficiency in those mice drives an altered resistance to stress. Indeed, mimicking GC-induced stress by DXM administration reveals here a slower thymus recovery in mutant mice, as demonstrated by MRI quantification of thymic volumes and sj/dβ ratio. To the best of our knowledge, this is the first time thymus regeneration after DXM-induced atrophy is longitudinally followed by MRI. This method was validated by Brooks and colleagues as a non-invasive way to measure thymus involution induced by DXM, giving high statistical power using less animals compared with measurement of tissue weight (35). However, considering the high variability observed in thymic volumes of control-injected mice and the small size of each group, results should be interpreted with caution. Moreover, thymus weight and cellularity as well as TREC number measured 1 day after the last MRI session showed normal values in mutant mice injected with DXM. Altogether, those results do not allow to firmly validate the stress hypothesis. Challenges with other type of stress, like infectious stress, are currently performed.

Aging is considered as a stressful situation for the immune system. It is well known that thymus undergo severe atrophy with aging, and elderly are less resistant to infections and autoimmune diseases (45). GH deficiency has been described to extend lifespan and delay immune aging (46, 47). For example, a study in Snell–Bagg and GHRH-R-deficient mice showed a 40% increased longevity regarded to WT mice and some parameters of aging immune system were also improved: similar proportion of memory cells and T cell function compared with young animals (47). This is consistent with the antagonistic pleiotropy theory, according to which genes conferring reproductive advantages are selected throughout evolution, despite their deleterious effects at long term (48). However, in our study, the somatotrope deficiency is not an aggravating factor for the aging immune system. Thymus atrophy, seen by the decreased weight and cellularity of the organ as well as TREC number, is parallel between mutant and normal mice. It should be pointed that our model is a genetic defect in GHRH that affect all the somatotrope axis since the beginning of development. The results should therefore be taken with precautions when comparing to acquired GH deficiency like it is postulated with aging.

Another surprising conclusion of this work is the inefficiency of GH supplementation to restore immune parameters, despite the clear metabolic effects of the treatment. Indeed, GH-daily injection in *Ghrh*KO mice results in increased body, spleen, and thymus weight and stimulation of IGF-1 production in the liver, as expected (16). However, none of the immune parameters analyzed, i.e., thymic and peripheral lymphocytes phenotype and TREC content, was modified by the 6-week-long treatment, even in old animals. This is surprising since numerous works showed that GH injection had beneficial effects on thymic function (3, 23), especially in aged rodents where it could reverse thymic involution (5) and on antibody production (37). Once again, stress hypothesis can explain the discrepancy between our results and literature. Another possibility is that IGF-1 is the main actor of somatotrope actions in the immune system (9, 49), and the dose of GH injected (1 mg/kg) was not able to induce the production of a sufficient amount of IGF-1. Indeed, IGF-1 was under detection limit of 4.0 ng/ml in serum of GH-injected *Ghrh*KO mice (data not shown).

Altogether, these data show that the severe somatotrope deficiency of *Ghrh*$^{-/-}$ mice essentially impacts the spleen and B compartment of the adaptive immune system, while it only marginally affects thymic function and T cell development. Our laboratory is now investigating the susceptibility of *Ghrh*$^{-/-}$ mice to T-independent and T-dependent pathogens.

ETHICS STATEMENT

This study was carried out in accordance with the European recommendations for animal health care and the protocols were approved by the Animal Ethics Committee of the University of Liege GIGA Institute.

AUTHOR CONTRIBUTIONS

GB and KF are equal first authors and performed all experiments. CR-C assisted technically GB and KF. GBe and AP performed MRI analyses. RS provided the laboratory with GHRH-KO mice. GB, GBe, AP, HM, and VG designed the protocol of experiments. GB, VG, and HM wrote the manuscript. VG and HM are equal last authors and supervised the whole experimental work.

FUNDING

This study was supported by grants from the "Fonds National de la Recherche Scientifique" (F.R.S.-FNRS; Belgium), the Fonds Léon Fredericq (Belgium), and the Federation Wallonia-Brussels (ARC Somasthym) for financial support. The authors thank Pr. R. Cheynier (Institut Cochin, Paris) for helping to set up TREC quantification and providing corresponding plasmids, L. Duwez, F. Olivier, and Pr. P. Drion from the GIGA Animal Facility for animal management and Dr. S. Ormanese and R. Stephan from the Cell Imaging and Flow Cytometry GIGA Technological Platform for their help for flow cytometry experiments design and analyses. We also thank Pfizer for kindly providing Genotonorm (human recombinant GH).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at https://www.frontiersin.org/articles/10.3389/fendo.2018.00296/full#supplementary-material.

REFERENCES

1. Bazan JF. Structural design and molecular evolution of a cytokine receptor superfamily. *Proc Natl Acad Sci U S A* (1990) 87:6934–8. doi:10.1073/pnas.87.18.6934
2. Baroni C. Thymus, peripheral lymphoid tissues and immunological responsiveness of the pituitary dwarf mouse. *Experientia* (1967) 23:282–3. doi:10.1007/BF02135688
3. Baroni CD, Fabris N, Bertoli G. Effects of hormones on development and function of lymphoid tissues. Synergistic action of thyroxin and somatotropic hormone in pituitary dwarf mice. *Immunology* (1969) 17:303–14.
4. Duquesnoy RJ. Immunodeficiency of the thymus-dependent system of the Ames dwarf mouse. *J Immunol* (1972) 108:1578–90.
5. Kelley KW, Brief S, Westly HJ, Novakofski J, Bechtel PJ, Simon J, et al. GH3 pituitary adenoma cells can reverse thymic aging in rats. *Proc Natl Acad Sci U S A* (1986) 83:5663–7. doi:10.1073/pnas.83.15.5663
6. Bodart G, Farhat K, Charlet-Renard C, Salvatori R, Geenen V, Martens H. The somatotrope growth hormone-releasing hormone/growth hormone/insulin-like growth factor-1 axis in immunoregulation and immunosenescence. *Front Horm Res* (2017) 48:147–59. doi:10.1159/000452913
7. Savino W, Dardenne M. Pleiotropic modulation of thymic functions by growth hormone: from physiology to therapy. *Curr Opin Pharmacol* (2010) 10:434–42. doi:10.1016/j.coph.2010.04.002
8. Napolitano LA, Schmidt D, Gotway MB, Ameli N, Filbert EL, Ng MM, et al. Growth hormone enhances thymic function in HIV-1-infected adults. *J Clin Invest* (2008) 118:1085–98. doi:10.1172/JCI32830
9. Morrhaye G, Kermani H, Legros JJ, Baron F, Beguin Y, Moutschen M, et al. Impact of growth hormone (GH) deficiency and GH replacement upon thymus function in adult patients. *PLoS One* (2009) 4:e5668. doi:10.1371/journal.pone.0005668
10. Dumont F, Robert F, Bischoff P. T and B lymphocytes in pituitary dwarf Snell-Bagg mice. *Immunology* (1979) 38:23–31.
11. Montecino-Rodriguez E, Clark RG, Powell-Braxton L, Dorshkind K. Primary B cell development is impaired in mice with defects of the pituitary/thyroid axis. *J Immunol* (1997) 159:2712–9.
12. Schneider GB. Immunological competence in Snell-Bagg pituitary dwarf mice: response to the contact-sensitizing agent oxazolone. *Am J Anat* (1976) 145:371–93. doi:10.1002/aja.1001450306
13. Dorshkind K, Horseman ND. The roles of prolactin, growth hormone, insulin-like growth factor-I, and thyroid hormones in lymphocyte development and function: insights from genetic models of hormone and hormone receptor deficiency. *Endocr Rev* (2000) 21:292–312. doi:10.1210/edrv.21.3.0397
14. Alba M, 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
15. Alba M, Fintini D, Bowers CY, Parlow AF, Salvatori R. Effects of long-term treatment with growth hormone-releasing peptide-2 in the GHRH knockout mouse. *Am J Physiol Endocrinol Metab* (2005) 289:E762–7. doi:10.1152/ajpendo.00203.2005
16. Alba M, Fintini D, Salvatori R. Effects of recombinant mouse growth hormone treatment on growth and body composition in GHRH knock out mice. *Growth Horm IGF Res* (2005) 15:275–82. doi:10.1016/j.ghir.2005.05.001
17. Alba M, Schally AV, Salvatori R. Partial reversibility of growth hormone (GH) deficiency in the GH-releasing hormone (GHRH) knockout mouse by postnatal treatment with a GHRH analog. *Endocrinology* (2005) 146:1506–13. doi:10.1210/en.2004-1044
18. Dulude G, Cheynier R, Gauchat D, Abdallah A, Kettaf N, Sekaly RP, et al. The magnitude of thymic output is genetically determined through controlled intrathymic precursor T cell proliferation. *J Immunol* (2008) 181:7818–24. doi:10.4049/jimmunol.181.11.7818
19. Kermani H, Goffinet L, Mottet M, Bodart G, Morrhaye G, Dardenne O, et al. Expression of the growth hormone/insulin-like growth factor axis during Balb/c thymus ontogeny and effects of growth hormone upon *ex vivo* T cell differentiation. *Neuroimmunomodulation* (2011) 18:137–47. doi:10.1159/000328844
20. Brooks KJ, Bunce KT, Haase MV, White A, Changani KK, Bate ST, et al. MRI quantification *in vivo* of corticosteroid induced thymus involution in mice: correlation with *ex vivo* measurements. *Steroids* (2005) 70:267–72. doi:10.1016/j.steroids.2004.11.013
21. Beckmann N, Gentsch C, Baumann D, Bruttel K, Vassout A, Schoeffter P, et al. Non-invasive, quantitative assessment of the anatomical phenotype of corticotropin-releasing factor-overexpressing mice by MRI. *NMR Biomed* (2001) 14:210–6. doi:10.1002/nbm.704
22. Barrat F, Lesourd BM, Louise A, Boulouis HJ, Vincent-Naulleau S, Thibault D, et al. Surface antigen expression in spleen cells of C57B1/6 mice during ageing: influence of sex and parity. *Clin Exp Immunol* (1997) 107:593–600. doi:10.1046/j.1365-2249.1997.3021199.x
23. Murphy WJ, Durum SK, Longo DL. Role of neuroendocrine hormones in murine T cell development. Growth hormone exerts thymopoietic effects *in vivo*. *J Immunol* (1992) 149:3851–7.
24. Montecino-Rodriguez E, Clark R, Johnson A, Collins L, Dorshkind K. Defective B cell development in Snell dwarf (dw/dw) mice can be corrected by thyroxine treatment. *J Immunol* (1996) 157:3334–40.
25. Oliveira CR, Salvatori R, Nobrega LM, Carvalho EO, Menezes M, Farias CT, et al. Sizes of abdominal organs in adults with severe short stature due to severe, untreated, congenital GH deficiency caused by a homozygous mutation in the GHRH receptor gene. *Clin Endocrinol (Oxf)* (2008) 69:153–8. doi:10.1111/j.1365-2265.2007.03148.x
26. Jardieu P, Clark R, Mortensen D, Dorshkind K. *In vivo* administration of insulin-like growth factor-I stimulates primary B lymphopoiesis and enhances lymphocyte recovery after bone marrow transplantation. *J Immunol* (1994) 152:4320–7.
27. Landreth KS, Narayanan R, Dorshkind K. Insulin-like growth factor-I regulates pro-B cell differentiation. *Blood* (1992) 80:1207–12.
28. SmaniottoS,Mendes-da-CruzDA,Carvalho-PintoCE,AraujoLM,DardenneM, Savino W. Combined role of extracellular matrix and chemokines on peripheral lymphocyte migration in growth hormone transgenic mice. *Brain Behav Immun* (2010) 24:451–61. doi:10.1016/j.bbi.2009.11.014
29. Sumita K, Hattori N, Inagaki C. Effects of growth hormone on the differentiation of mouse B-lymphoid precursors. *J Pharmacol Sci* (2005) 97:408–16. doi:10.1254/jphs.FPJ04054X
30. Gagnerault MC, Postel-Vinay MC, Dardenne M. Expression of growth hormone receptors in murine lymphoid cells analyzed by flow cytofluorometry. *Endocrinology* (1996) 137:1719–26. doi:10.1210/endo.137.5.8612507
31. Clark R, Strasser J, McCabe S, Robbins K, Jardieu P. Insulin-like growth factor-1 stimulation of lymphopoiesis. *J Clin Invest* (1993) 92:540–8. doi:10.1172/JCI116621

32. Chu YW, Schmitz S, Choudhury B, Telford W, Kapoor V, Garfield S, et al. Exogenous insulin-like growth factor 1 enhances thymopoiesis predominantly through thymic epithelial cell expansion. *Blood* (2008) 112:2836–46. doi:10.1182/blood-2008-04-149435
33. Postel-Vinay MC, de Mello Coelho V, Gagnerault MC, Dardenne M. Growth hormone stimulates the proliferation of activated mouse T lymphocytes. *Endocrinology* (1997) 138:1816–20. doi:10.1210/endo.138.5.5108
34. Shohreh R, Pardo CA, Guaraldi F, Schally AV, Salvatori R. GH, but not GHRH, plays a role in the development of experimental autoimmune encephalomyelitis. *Endocrinology* (2011) 152:3803–10. doi:10.1210/en.2011-1317
35. Foster MP, Jensen ER, Montecino-Rodriguez E, Leathers H, Horseman N, Dorshkind K. Humoral and cell-mediated immunity in mice with genetic deficiencies of prolactin, growth hormone, insulin-like growth factor-I, and thyroid hormone. *Clin Immunol* (2000) 96:140–9. doi:10.1006/clim.2000.4889
36. Baroni C. Mouse thymus in hereditary pituitary dwarfism. *Acta Anat (Basel)* (1967) 68:361–73. doi:10.1159/000143040
37. Pierpaoli W, Baroni C, Fabris N, Sorkin E. Hormones and immunological capacity. II. Reconstitution of antibody production in hormonally deficient mice by somatotropic hormone, thyrotropic hormone and thyroxin. *Immunology* (1969) 16:217–30.
38. Dorshkind K, Welniak L, Gault RA, Hixon J, Montecino-Rodriguez E, Horseman ND, et al. Effects of housing on the thymic deficiency in dwarf mice and its reversal by growth hormone administration. *Clin Immunol* (2003) 109:197–202. doi:10.1016/S1521-6616(03)00181-5
39. Chatterton RT Jr, Murray CL, Hellman L. Endocrine effects on leukocytopoiesis in the rat. I. Evidence for growth hormone secretion as the leukocytopoietic stimulus following acute cortisol-induced lymphopenia. *Endocrinology* (1973) 92:775–87. doi:10.1210/endo-92-3-775
40. Kelley KW, Dantzer R. Growth hormone and prolactin as natural antagonists of glucocorticoids in immunoregulation. In: Plotnikoff NP, Murgo A, Faith R, Wybran J, editors. *Stress and Immunity*. Boca Raton, Florida: CRC Press (1991). p. 433–51.
41. Moutoussamy S, Kelly PA, Finidori J. Growth-hormone-receptor and cytokine-receptor-family signaling. *Eur J Biochem* (1998) 255:1–11. doi:10.1046/j.1432-1327.1998.2550001.x
42. Stocklin E, Wissler M, Gouilleux F, Groner B. Functional interactions between Stat5 and the glucocorticoid receptor. *Nature* (1996) 383:726–8. doi:10.1038/383726a0
43. Krishnan N, Thellin O, Buckley DJ, Horseman ND, Buckley AR. Prolactin suppresses glucocorticoid-induced thymocyte apoptosis *in vivo*. *Endocrinology* (2003) 144:2102–10. doi:10.1210/en.2003-0053
44. Lepletier A, de Carvalho VF, Rodrigues e Silva PM, Villar S, Perez AR, Savino W, et al. *Trypanosoma cruzi* disrupts thymic homeostasis by altering intrathymic and systemic stress-related endocrine circuitries. *PLoS Negl Trop Dis* (2013) 7:e2470. doi:10.1371/journal.pntd.0002470
45. Palmer DB. The effect of age on thymic function. *Front Immunol* (2013) 4:316. doi:10.3389/fimmu.2013.00316
46. Bartke A, Sun LY, Longo V. Somatotropic signaling: trade-offs between growth, reproductive development, and longevity. *Physiol Rev* (2013) 93:571–98. doi:10.1152/physrev.00006.2012
47. Flurkey K, Papaconstantinou J, Miller RA, Harrison DE. Lifespan extension and delayed immune and collagen aging in mutant mice with defects in growth hormone production. *Proc Natl Acad Sci U S A* (2001) 98:6736–41. doi:10.1073/pnas.111158898
48. Williams GC. Pleiotropy, natural selection, and the evolution of senescence. *Evolution* (1957) 11:398–411. doi:10.1111/j.1558-5646.1957.tb02911.x
49. Yamada M, Hato F, Kinoshita Y, Tominaga K, Tsuji Y. The indirect participation of growth hormone in the thymocyte proliferation system. *Cell Mol Biol (Noisy-le-grand)* (1994) 40:111–21.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

MINI REVIEW
published: 30 January 2018
doi: 10.3389/fendo.2018.00013

Intrinsic and Extrinsic Thymic Adrenergic Networks: Sex Steroid-Dependent Plasticity

Gordana Momčilo Leposavić[1]* *and Ivan M. Pilipović*[2]

[1] *Department of Physiology, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia,* [2] *Immunology Research Centre "Branislav Janković", Institute of Virology, Vaccines and Sera "Torlak", Belgrade, Serbia*

The thymus is sexually differentiated organ providing microenvironment for T-cell precursor differentiation/maturation in the major histocompatibility complex-restricted self-tolerant T cells. With increasing age, the thymus undergoes involution leading to the decline in efficacy of thymopoiesis. Noradrenaline from thymic nerve fibers and "(nor) adrenergic" cells is involved in the regulation of thymopoiesis. In rodents, noradrenaline concentration in thymus and adrenoceptor (AR) expression on thymic cells depend on sex and age. These differences are suggested to be implicated in the development of sexual diergism and the age-related decline in thymopoiesis. The programming of both thymic sexual differentiation and its involution occurs during the critical early perinatal period and may be reprogrammed during peripubertal development. The thymic (re) programming is critically dependent on circulating levels of gonadal steroids. Although the underlying molecular mechanisms have not yet been elucidated fully, it is assumed that the gonadal steroid action during the critical perinatal/peripubertal developmental periods leads to long-lasting changes in the efficacy of thymopoiesis partly through (re) programming of "(nor)adrenergic" cell networks and AR expression on thymic cells.

Keywords: thymic noradrenergic innervation, noradrenaline-synthesizing thymic cells, adrenoceptors, sex steroids, thymic involution, thymic programming/reprogramming

OPEN ACCESS

Edited by:
Daniella Areas Mendes-Da-Cruz, Fundação Oswaldo Cruz (Fiocruz), Brazil

Reviewed by:
Arturo Ortega, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), Mexico
Wei-Jiang Zhao, Shantou University, China

***Correspondence:**
Gordana Momčilo Leposavić
gordana.leposavic@pharmacy.bg.ac.rs

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: *22 November 2017*
Accepted: *11 January 2018*
Published: *30 January 2018*

Citation:
Leposavić GM and Pilipović IM (2018) Intrinsic and Extrinsic Thymic Adrenergic Networks: Sex Steroid-Dependent Plasticity. Front. Endocrinol. 9:13. doi: 10.3389/fendo.2018.00013

The thymus is organ in which T cells are continually generated in a highly dynamic process comprising T-cell receptor (TCR) gene rearrangement, lineage commitment, and selection (1). These processes are linked to distinct rates of proliferation and cell death by apoptosis (1). With increasing age, the thymus atrophies and declines in functions, the phenomenon termed involution (2). Consequently, thymic generation of naïve T cells declines (2, 3). This leads to the shrinkage of peripheral TCR repertoire and the expansion of memory T cell compartment, i.e., to the changes covered by the canopy term immunosenescence (3–5). At the clinical level, the immunosenescence is associated with a greater susceptibility to infections (6, 7), an impaired response to vaccinations (8, 9), and an increased propensity for malignant diseases (10, 11). In addition, according to the U.S. Center for Disease Control, approximately 80% of aged individuals are afflicted with at least one chronic disease as a result of a declination of immune function. Consequently, factors contributing to the thymic involution and mechanisms of their action are becoming the subject of increased interest in the scientific and healthcare communities alike. It should be emphasized that understanding of the mechanisms underlying thymic involution is important not only for moderating the deleterious effects of immunosenescence, but also for envisaging strategies to "rejuvenate" the immune system. It is noteworthy that even in a significant thymic involution thymopoiesis does not cease completely, so it may be enhanced (12). The thymic "rejuvenation" becomes particularly important after exposure

to chemotherapy, ionizing radiation, and some infective agents (e.g., HIV-1) (13).

There is evidence that (i) early perinatal programming of the thymus is crucial for the development of thymic involution, and consequently the efficacy of immune responses from early life through adulthood (14), and (ii) this phenomenon is sexually dimorphic (14, 15). Consistently, sex differences in the organ size, structural organization, and thymopoiesis (14–22), and consequently T-cell immune response (23, 24), have been observed. A more rapid thymic involution was found in male compared with female mice (25). Consequently, adult females have the ability to reject allografts more efficiently, a greater ability to combat various viral and bacterial infections, and superior antitumor responses (23).

There are data indicating that the early programming of the thymus development/involution is shaped by genetic, environmental, and hormonal factors (14). The role of genetic factors has been shown in both mice and rats (26–31). These genetically based differences are suggested to be connected to strain differences in susceptibility to various pathologies involving immune mechanisms (26–30). Environmental factors, such as malnutrition, and exposure to endocrine disruptors, in early postnatal life are also shown to influence the pace of thymic involution (32). Alterations in circulating levels of sex steroids in the critical early postnatal developmental "window" may influence not only sexual dimorphism in structural and functional thymic parameters, but also the timing of thymic involution (15, 21, 33). Furthermore, gonadal steroids may influence sexual dimorphism in thymopoiesis, and the age-related decline in its efficacy through: (i) modulating thymic extrinsic (encompassing noradrenergic nerve fibers) and intrinsic [composed of noradrenaline-synthesizing cells, i.e., "(nor)adrenergic" cells] adrenergic regulatory networks, in terms of their density/noradrenaline content and (ii) adrenoceptor (AR) expression on thymic cells (34, 35). In addition, it should be pointed out that the ablation of gonadal steroids during the peripubertal developmental "window" leads not only to short-term increase in thymic weight and enhancement of thymopoiesis, but also to the long-lasting thymic "rejuvenation" (33).

The central goal of this mini review is to summarize recent findings and current knowledge related to the mechanisms of indirect (nor)adrenaline-mediated action of gonadal steroids on the programming/reprogramming of thymic involution, as its action may be easily controlled by many drugs in use for non-immune indications.

THYMIC EXTRINSIC AND INTRINSIC (NOR)ADRENERGIC REGULATORY NETWORKS

Thymic Extrinsic (Nor)adrenergic Network

The thymus receives extensive noradrenergic innervation (36, 37). The varicose noradrenergic fibers terminate in close proximity to thymocytes (37, 38), and various subsets of thymic non-lymphoid (stromal) cells (38–41). In rodents, noradrenergic fibers appear in the thymus in late embryonic period, and their density increases during prepubertal development (42, 43). The data on postpubertal changes in their density are inconsistent (44–50). In advanced age, in rodents of distinct (sub)strains has been observed decrease, increase and lack of changes in thymic noradrenergic nerve fiber density compared with young adult (sub)strain-matched ones (44–50). This inconsistency is most likely linked to (sub)strain and sex-dependent differences in the kinetics of postpubertal changes in thymic noradrenergic innervation. It has also been suggested that the noradrenaline content in thymic nerve fibers, and consequently thymic noradrenaline concentration vary with age (44–50). In addition, both thymic parameters were found to be greater in male than in age-matched female rats (51) (**Figure 1**).

Thymic Intrinsic (Nor)adrenergic Network

Many types of mature immune cells synthesize and secrete catecholamines (52–54). The investigations of the expression of tyrosine hydroxylase (TH), the key rate-limiting enzyme in catecholamine synthesis in freshly isolated thymic cells, cultured thymocytes and cells from adult thymic organ culture revealed that thymic cells, including thymocytes, synthetize noradrenaline (34, 51, 55). TH-immunoreactive cells were found across all thymocyte subsets delineated by CD3 expression levels, but their frequency was highest among the most mature $CD3^{high}$ thymocytes (51). In addition, TH-immunoreactive cells were observed in various thymic non-lymphoid cell subpopulations (44, 51). Their density varies across distinct thymic microenvironments. They are frequent at the medullary side of the corticomedullary junction, whereas their density is moderate and poor in the subcapsular cortex, and intracortically/intramedullary, respectively (51). This is important as various thymic non-lymphoid cell subsets are strategically positioned in particular thymic microenvironments to orchestrate thymocyte differentiation/maturation (56). TH immunoreactivity was observed in thymic epithelial cells (TECs) (39, 51, 57–59), macrophages, and dendritic cells (44, 60). In TEC population, TH immunoreactivity was found in neural crest-derived thymic nurse cells (51, 57, 58), type 1 (subcapsular/perivascular), and type 5 (located mainly in corticomedullary region) cells (39, 51, 59). The density of both lymphoid and non-lymphoid TH-immunoreactive cells was shown to be higher in male than in female rats (51) (**Figure 1**). In addition, the overall noradrenaline content in thymocytes was found to be greater in male compared with female adult rats (51) (**Figure 1**). Although studies in rat adult thymic organ and thymocyte cultures suggested that noradrenaline from thymic "(nor)adrenergic" cells is implicated in the fine tuning of thymopoisesis (55), a role for thymic intrinsic adrenergic network in thymic homeostasis is still far from being understood. It is noteworthy that intrinsic (nor)adrenergic cellular networks: (i) have also been identified in some other tissues and (ii) suggested to be particularly important under specific conditions, e.g., following sympathectomy, gonadectomy, chronic stress (45, 61–65), as it allows for mainly local regulation of the catecholamine influence (64, 66).

AR Expression on Thymic Cells

To corroborate modulatory role for noradrenaline in the thymus is the expression of ARs on both thymocytes and thymic

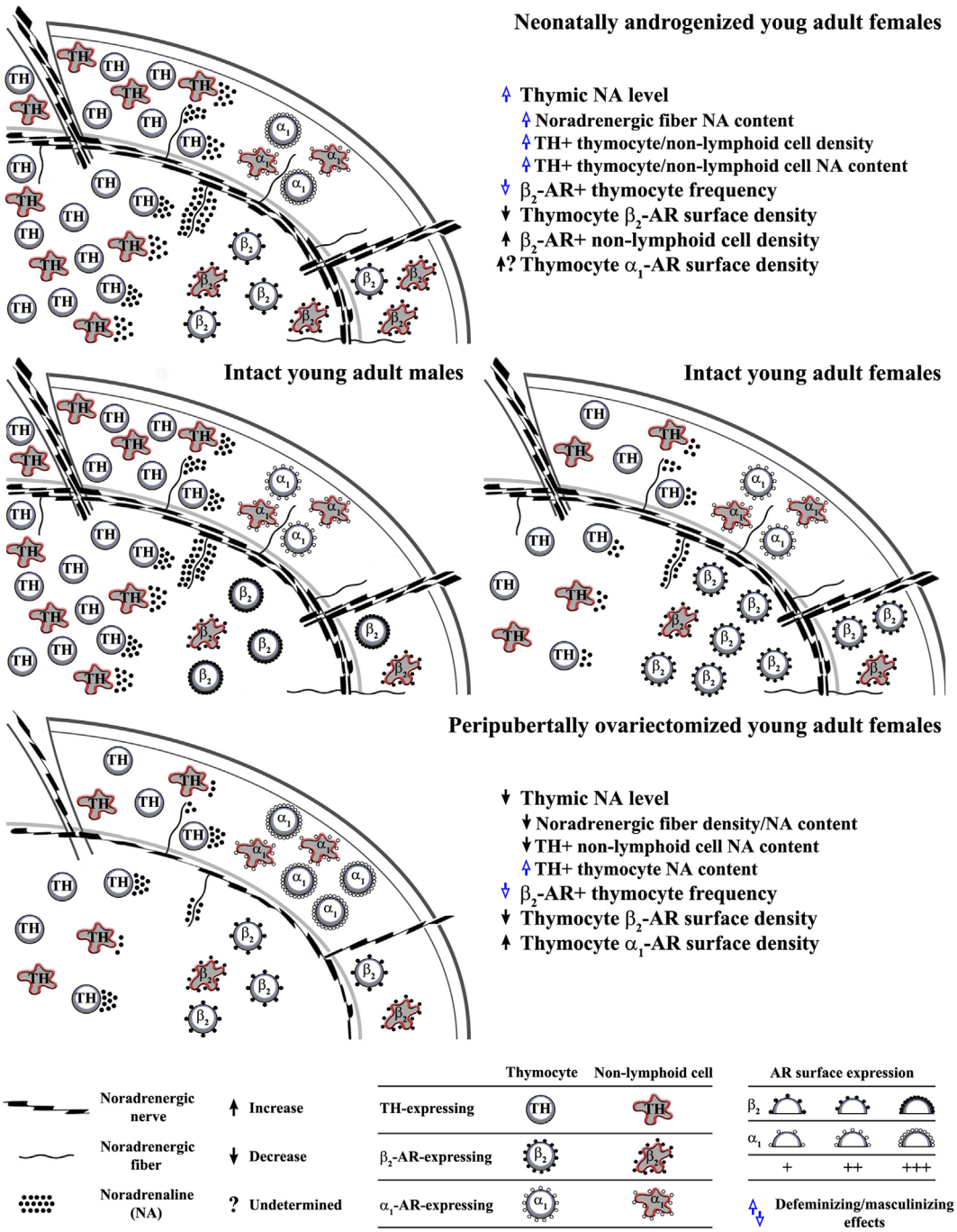

FIGURE 1 | Influence of alterations in circulating ovarian steroid levels in critical developmental periods on programming/reprogramming of thymic extrinsic and intrinsic adrenergic networks. This figure indicates (middle schemes) sex differences in noradrenaline content in noradrenergic nerve fibers and thymocytes, density of tyrosine hydroxylase (TH)-expressing ("adrenergic") cells, density of β_2-adrenoceptor (AR)$^+$ thymic cells and thymocyte β_2-AR surface density in young adult rat thymus, and influence of (upper scheme) single injection of testosterone on the third postnatal day to female rats and (lower scheme) ovariectomy in peripubertal period on noradrenergic nerve fiber and thymic "adrenergic" cell density and their noradrenaline content, as well as the density of AR-expressing thymic cells and thymocyte AR surface density in young adult rats.

non-lymphoid cells. Thymic cells express β_2- and α_1-AR (67–70). Their expression is reciprocally regulated during thymocyte maturation (50, 71, 72). The most mature $CD3^{high}$ thymocytes predominantly express β_2-AR, whereas α_1-AR expression is predominant on the most immature $CD3^-$ thymocytes (50, 60, 70, 72). There is sexual diergism in the expression of β_2-AR on thymocytes. Immunophenotyping showed the higher frequency of β_2-AR-expressing cells among thymocytes from female compared

with male young adult rats, but lower density of the receptor on their surface (73) (**Figure 1**). In addition, autoradiographic studies indicated a sexually dimorphic pattern of postnatal changes in the density of β-AR in rat thymus (69). There are no data on sex differences in α_1-AR expression on thymocytes.

The expression of β_2-AR was also demonstrated on cortical (aminopeptidase A^+), and medullary (UEA-1^+) TECs, $CD68^+$ macrophages, and $OX62^+$ dendritic cells (44). In addition, α_1-AR-immunoreactive cells were observed among TECs and macrophages located predominantly in subcapsular/subtrabecular and corticomedullary thymic regions (60). Thymic dendritic cells also express α_1-AR (74). The subsets of β_2-AR^+ and α_1-AR^+ non-lymphoid cells were shown to co-express TH (60). Thus, not only paracrine, but also autocrine noradrenaline action may be expected in the thymus.

GONADAL STEROIDS AND PROGRAMMING/REPROGRAMMING OF THE THYMIC (NOR)ADRENERGIC NETWORKS AND AR EXPRESSION

Early Postnatal Thymic Programming

The thymus is sexually differentiated organ (15). The sexual differentiation in the thymus, as in the brain areas controlling gonadotropin release, occurs during the critical perinatal period, and is governed by sex steroid-dependent mechanisms (15). In addition, the widely accepted organizational/activational hypotesis of the bran development is extended to encompass the thymic differentiation (15). According to the original hypothesis, in the absence of testicular androgens during the critical period (starting at the late prenatal period and continuing, at least, to day 5 postpartum), the areas controlling gonadotropin release develop in a primarily female manner (75–78). Conversely, the presence of testicular androgens leads to their defeminization/masculinization, a phenomenon known as neonatal androgenization (77–79). This postpones sexual maturation and leads to development of non-ovulatory ovaries with estrogen hyporesponsiveness (78, 80, 81). The mechanisms of testosterone action in the brain and thymus are extremely complex, as in both organs it converts into estrogen (15, 75–78), and consequently does not act only through androgen receptors (82). The binding of estradiol to classical estrogen receptor (ER)α or ERβ in the cytoplasm of target cells causes the receptor dimerization and translocation in nucleus, where the dimer associates with various coactivators to enable binding to the estrogen response elements (EREs) in or near the promoters of target genes (83). Estradiol can also influence expression of genes that do not harbor EREs in their promoter regions. In this case, ligand-activated ERs do not bind DNA directly, but through protein–protein interactions with other classes of transcription factors at their respective response elements in promotor region of their target genes (84). In addition, estradiol may act through membrane G protein-coupled ER (GPER, previously termed GPR30) (84). This involves mobilization of diverse signaling pathways and may depend on a number of conditions, like the availability of signal transduction molecules and downstream targets (84).

It was shown that a single injection of testosterone on the third postnatal day enhanced thymic growth and postponed thymic involution in female rats, which normally starts around puberty (85, 86). Accordingly, long-lasting changes in thymopoiesis, mirrored in the enhanced thymocyte differentiation/maturation in adult animals were observed (86). In addition, neonatal androgenization facilitated the generation of $CD4^-CD8^+TCR\alpha\beta^{high}$ cells, and consequently shifted $CD4^+/CD8^+$ recent thymic emigrant ratio in peripheral blood toward the latter (86). The thymopoietic changes were ascribed to thymocyte overexpression of Thy-1, as its overexpression reduces thymocyte negative selection and favors maturation of $CD8^+$ T cells (87). Considering $CD8^+$ T cell dominance in the periphery of males when compared with females (23, 88), the previous findings indicate defeminization/masculinization of T-cell compartment in adult neonatally androgenized rats, i.e., speak in favor of a sex steroid role in the sexual differentiation of thymus.

Although aware of the complexity of changes in neuroendocrine-thymic communications in neonatally androgenized rats, in this review we focused on those mediated by catecholamines. Neonatal androgenization was shown to increase thymic noradrenaline concentration in adult rats (35). This mainly reflected the increase in nerve fiber noradrenaline content (35). Consistent with the so-called transsynaptic action of sex steroids on neurotransmitter synthesis (89), the previous finding may be explained by an augmented sympathetic tone in neonatally androgenized rats (90, 91). However, the higher noradrenaline concentration partly reflected the greater density of noradrenaline-synthesizing cells and noradrenaline content per cell (35) (**Figure 1**). Considering that the circulating level of testosterone was elevated in neonatally androgenized rats (35), this could be associated with data indicating that androgens prominently transactivate TH promoter (92). In light of data from other studies (51), the previous findings suggest thymic defeminization/masculinization in neonatally androgenized rats (**Figure 1**).

As additional sign of defeminization/masculinization (73), the frequency of β_2-AR-expressing cells within thymocytes (35) was diminished in neonatally androgenized rats (**Figure 1**). In addition, neonatal androgenization decreased β_2-AR density on thymocytes (35) (**Figure 1**). Given that in many cell types estrogen, acting through classical ERs, upregulates β_2-AR expression (93, 94), the alterations in β_2-AR density could reflect estrogen hyporesponsiveness (80, 95). This hyporesponsiveness most likely emerged from the ER interaction with an excess of estrogen (as a result of testosterone aromatization) during the critical period (96, 97). The interaction of receptor with excess ligand in the critical period is shown to cause misprinting substantiated in diminished receptor binding capacity and responsivity in later life (96, 97). The elevation of thymic noradrenaline concentration following the testosterone injection could also impair the efficacy of β_2-AR signaling (through the hormonal misprinting) (35), leading to the diminished noradrenaline action as the ultimate effect. In favor of this assumption is the increase in Thy-1 expression in adult neonatally androgenized rats (83). Namely, the incubation of murine thymocytes with noradrenaline causes time- and concentration-dependent decreases in the Thy-1 mRNA levels, which

are completely preventable by propranolol (98, 99). Moreover, given that: (i) noradrenaline upregulates α_1-AR expression (100) and (ii) long-lasting α_1-AR blockade facilitates thymocyte differentiation/maturation toward $CD4^+CD8^-TCR\alpha\beta^{high}$ cells (70), the contribution of an augmented α_1-AR signaling (reflecting its increased density and/or noradrenaline concentration) to the thymocyte maturation skewed toward $CD4^-CD8^+$ $TCR\alpha\beta^{high}$ cells in adult neonatally androgenized rats cannot be ruled out.

In favor of the role of sex steroids in perinatal programming of thymic noradrenergic networks are also data showing that orchidectomy in the critical perinatal period lowers levels of both neurally- and thymocyte-derived noradrenaline in adult rats and thereby contributes to the deceleration of the thymic involution (34). This is consistent with data indicating that not only in presence of excess ligand in the critical periods, but also in its absence the ligand–receptor connection changes for life (101).

To summarize, the previous findings indicate that alterations in circulating levels of sex steroids in the critical perinatal period may affect the programming of the sexually dimorphic (nor) adrenaline influence on thymopoiesis. However, the molecular mechanisms standing behind this phenomenon remain to be elucidated.

Peripubertal Thymic Reprogramming

It has been suggested that the hormonal changes occurring at the time of puberty lay the framework for biological differences that persist throughout life (102). In addition, the original organizational/activational hypothesis of sexual differentiation of the brain has been extended to include puberty (76, 103). Namely, ovariectomy in peripubertal period leads to a long-lasting postponement/alleviation of the postpubertal decline in thymopoiesis (33, 104). This could be partly related to ovariectomy-induced changes in thymocyte proliferation (35). Given that the age-related decline in thymopoiesis has been partly related to the rise in the thymic noradrenaline level (44, 50, 105), one may assume that the peripubertal ovariectomy affects thymic adrenergic networks. Indeed, it was shown that it diminishes the thymic noradrenaline level in young adult (2-month-old) rats (45). This reflected the decrease in the density of noradrenergic nerve fibers and noradrenaline content in both noradrenergic nerve fibers and non-lymphoid cells, as thymocyte noradrenaline content increased (45) (**Figure 1**). These changes were preventable by estrogen supplementation (45). This could be explained by the following facts: (i) estrogen represents the key factor in remodeling of noradrenergic innervation in some other tissues (106) and (ii) is implicated in the regulation of TH expression (107). Estrogen is suggested to regulate TH gene expression through direct genomic effects, as the TH promoter contains several elements, including the activation protein 1 and Sp1/Egr1 motifs that might mediate estrogen action on TH gene (108, 109). The thymic cell type-specific effects of peripubertal ovariectomy on TH expression could be explained by data indicating that estrogen may regulate TH transcription in opposite direction through ERα and ERβ (110). Given that estrogen may influence TH expression trough extragenomic and indirect genomic effects, it may also be supposed that estrogen, through the same ER, may produce opposing effects by interacting with proteins with distinct action on gene transcription in distinct cells (111, 112). In peripubertally ovariectomized rats, the density of noradrenergic nerve fibers and TH-expressing non-lymphoid cells remained lower than in age-matched controls until the age of 11 months (45). On the other hand, thymocyte noradrenaline, which was elevated in 2-month-old peripubertally ovariectomized rats, continued to rise until the age of 11 months (45). In 11-month-old peripubertally ovariectomized rats it was comparable with controls (45). Thus, it seems that the ovariectomy-induced changes are long lasting (45).

In addition, peripubertal ovariectomy in young adult rats diminished the average thymocyte surface density of β_2-AR, but it increased that of α_1-AR (reflecting estrogen, and estrogen and progesterone deficiency, respectively) (45) (**Figure 1**). These changes, despite the rise in circulating estrogen level post-ovariectomy because of extragonadal synthesis (113), remained stable until the age of 11 months (45). This could be related to a decreased sensitivity to estrogen action, as a consequence of peripubertal hormone misprinting. Finally, it is noteworthy that the increased noradrenaline content in thymocytes and diminished frequency of β_2-AR^+ thymocytes in young adult ovariectomized rats suggested that peripubertal ovariectomy instigates some signs of thymic defeminization/masculinization (51, 73) (**Figure 1**).

The putative role of peripubertal orchidectomy in long-lasting reprogramming of the thymic adrenergic networks has not been examined. However, 1 month following peripubertal orchidectomy the changes in both extrinsic and intrinsic noradrenergic networks were similar to those described 1 month following ovariectomy in the same age (44, 45). In addition, an impaired β-AR-mediated influence on thymus led to more efficient thymocyte positive selection/less efficient negative selection, and preferential differentiation/maturation of thymocytes into mature $CD4^+CD8^-TCR\alpha\beta^{high}$ cells in orchidectomized rats (44), i.e., to a more "feminine" pattern of T-cell development (23).

CONCLUSION

In summary, a growing body of evidence indicates that both thymic sexual differentiation and involution are, at least partly, "controlled" during the critical developmental periods by gonadal steroids. In addition, it suggests that the gonadal steroid-mediated thymic (re)programming involves extrinsic and intrinsic noradrenergic regulatory networks and AR expression on thymic cells. The challenge remains to elucidate the molecular mechanisms underlying these gonadal steroid-induced effects. Nonetheless, it may be assumed that (i) alterations in circulating levels of gonadal steroids during the critical developmental periods (either induced endogenously or by endocrine disruptors in the environment) lead to long-lasting effects on thymopoiesis and (ii) pharmacological manipulation with (nor)adrenaline action on thymus may be useful means in preventing/moderating deleterious effects of aging on thymopoiesis.

AUTHOR CONTRIBUTIONS

GL wrote the draft version, and IP designed the figure, whereas GL and IP equally contributed to the editing of this manuscript.

FUNDING

The study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant No. 175050).

REFERENCES

1. Krueger A, Ziętara N, Łyszkiewicz M. T cell development by the numbers. *Trends Immunol* (2016) 38:128–39. doi:10.1016/j.it.2016.10.007
2. Rezzani R, Nardo L, Favero G, Peroni M, Rodella LF. Thymus and aging: morphological, radiological, and functional overview. *Age (Dordr)* (2014) 36:313–51. doi:10.1007/s11357-013-9564-5
3. Aw D, Palmer DB. It's not all equal: a multiphasic theory of thymic involution. *Biogerontology* (2012) 13:77–81. doi:10.1007/s10522-011-9349-0
4. Linton PJ, Dorshkind K. Age-related changes in lymphocyte development and function. *Nat Immunol* (2004) 5:133–9. doi:10.1038/ni1033
5. Goronzy JJ, Lee WW, Weyand CM. Aging and T-cell diversity. *Exp Gerontol* (2007) 42:400–6. doi:10.1016/j.exger.2006.11.016
6. Yager EJ, Ahmed M, Lanzer K, Randall TD, Woodland DL, Blackman MA. Age-associated decline in T cell repertoire diversity leads to holes in the repertoire and impaired immunity to influenza virus. *J Exp Med* (2008) 205:711–23. doi:10.1084/jem.20071140
7. Nikolich-Žugich J. Ageing and life-long maintenance of T-cell subsets in the face of latent persistent infections. *Nat Rev Immunol* (2008) 8:512–22. doi:10.1038/nri2318
8. Haynes L, Swain SL. Why aging T cells fail: implications for vaccination. *Immunity* (2006) 24:663–6. doi:10.1016/j.immuni.2006.06.003
9. Giefing-Kröll C, Berger P, Lepperdinger G, Grubeck-Loebenstein B. How sex and age affect immune responses, susceptibility to infections, and response to vaccination. *Aging Cell* (2015) 14:309–21. doi:10.1111/acel.12326
10. Fulop T, Kotb R, Fortin CF, Pawelec G, de Angelis F, Larbi A. Potential role of immunosenescence in cancer development. *Ann N Y Acad Sci* (2010) 1197:158–65. doi:10.1111/j.1749-6632.2009.05370.x
11. Foster AD, Sivarapatna A, Gress RE. The aging immune system and its relationship with cancer. *Aging Health* (2011) 7:707–18. doi:10.2217/ahe.11.56
12. Mackall CL, Punt JA, Morgan P, Farr AG, Gress RE. Thymic function in young/old chimeras: substantial thymic T cell regenerative capacity despite irreversible age-associated thymic involution. *Eur J Immunol* (1998) 28:1886–93. doi:10.1002/(SICI)1521-4141(199806)28:06<1886::AID-IMMU1886>3.0.CO;2-M
13. Lynch HE, Goldberg GL, Chidgey A, Van den Brink MR, Boyd R, Sempowski GD. Thymic involution and immune reconstitution. *Trends Immunol* (2009) 30:366–73. doi:10.1016/j.it.2009.04.003
14. Gui J, Mustachio LM, Su DM, Craig RW. Thymus size and age-related thymic involution: early programming, sexual dimorphism, progenitors and stroma. *Aging Dis* (2012) 3:280–90.
15. de Fougerolles Nunn E, Greenstein B, Khamashta M, Hughes GR. Evidence for sexual dimorphism of estrogen receptors in hypothalamus and thymus of neonatal and immature Wistar rats. *Int J Immunopharmacol* (1999) 21:869–77. doi:10.1016/S0192-0561(99)00055-7
16. Leposavić G, Karapetrović B, Budeč M, Kosec D. Sex differences in the phenotypic characteristics of rat thymocytes. *Acta Vet (Beogr)* (1995) 45:205–10.
17. Leposavić G, Karapetrović B, Obradović S, Vidić Danković B, Kosec D. Differential effects of gonadectomy on the thymocyte phenotypic profile in male and female rats. *Pharmacol Biochem Behav* (1996) 54:269–76. doi:10.1016/0091-3057(95)02165-5
18. Pido-Lopez J, Imami N, Aspinall R. Both age and gender affect thymic output: more recent thymic migrants in females than males as they age. *Clin Exp Immunol* (2001) 125:409–13. doi:10.1046/j.1365-2249.2001.01640.x
19. Gubbels Bupp MR. Sex, the aging immune system, and chronic disease. *Cell Immunol* (2015) 294:102–10. doi:10.1016/j.cellimm.2015.02.002
20. Dumont-Lagacé M, St-Pierre C, Perreault C. Sex hormones have pervasive effects on thymic epithelial cells. *Sci Rep* (2015) 5:12895. doi:10.1038/srep12895
21. Nagakubo D, Krauth B, Boehm T. Genetic and non-genetic determinants of thymic epithelial cell number and function. *Sci Rep* (2017) 7:10314. doi:10.1038/s41598-017-10746-8
22. Mitchell WA, Lang PO, Aspinall R. Tracing thymic output in older individuals. *Clin Exp Immunol* (2010) 161:497–503. doi:10.1111/j.1365-2249.2010.04209.x
23. Olsen NJ, Kovacs WJ. Gonadal steroids and immunity. *Endocr Rev* (1996) 17:369–84. doi:10.1210/edrv-17-4-369
24. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol* (2016) 16:626–38. doi:10.1038/nri.2016.90
25. Aspinall R, Andrew D. Gender-related differences in the rates of age associated thymic atrophy. *Dev Immunol* (2001) 8:95–106. doi:10.1155/2001/17064
26. Saito M, Amo H, Murakumo Y, Kojima A, Katoh H, Hiromatsu T, et al. Genetically determined thymus enlargement in the early life in BUF/Mna rats. *Tohoku J Exp Med* (1997) 182:309–18. doi:10.1620/tjem.182.309
27. Belyaev DK, Gruntenko EV. Strain differences in thymus weight in mice with different predispositions to spontaneous mammary cancer. *Nature* (1972) 237:401–2. doi:10.1038/237401a0
28. Peleg L, Nesbitt MN. Genetic control of thymus size in inbred mice. *J Hered* (1984) 75:126–30. doi:10.1093/oxfordjournals.jhered.a109886
29. Hsu HC, Zhang HG, Li L, Yi N, Yang PA, Wu Q, et al. Age-related thymic involution in C57BL/6J x DBA/2J recombinant-inbred mice maps to mouse chromosomes 9 and 10. *Genes Immun* (2003) 4:402–10. doi:10.1038/sj.gene.6363982
30. Wang X, Hsu HC, Wang Y, Edwards CK, Yang P, Wu Q, et al. Phenotype of genetically regulated thymic involution in young BXD RI strains of mice. *Scand J Immunol* (2006) 64:287–94. doi:10.1111/j.1365-3083.2006.01813.x
31. Gui J, Morales AJ, Maxey SE, Bessette KA, Ratcliffe NR, Kelly JA, et al. MCL1 increases primitive thymocyte viability in female mice and promotes thymic expansion into adulthood. *Int Immunol* (2011) 23:647–59. doi:10.1093/intimm/dxr073
32. Calder PC, Krauss-Etschmann S, de Jong EC, Dupont C, Frick JS, Frokiaer H, et al. Early nutrition and immunity-progress and perspectives. *Br J Nutr* (2006) 96:774–90. doi:10.1079/BJN20061881
33. Leposavić G, Perisić M. Age-associated remodeling of thymopoiesis: role for gonadal hormones and catecholamines. *Neuroimmunomodulation* (2008) 15:290–322. doi:10.1159/000156473
34. Leposavić G, Radojević K, Vidić-Danković B, Kosec D, Pilipović I, Perisić M. Early postnatal castration affects thymic and thymocyte noradrenaline levels and beta-adrenoceptor-mediated influence on the thymopoiesis in adult rats. *J Neuroimmunol* (2007) 182:100–15. doi:10.1016/j.jneuroim.2006.10.004
35. Radojević K, Kosec D, Perišić M, Pilipović I, Vidić-Danković B, Leposavić G. Neonatal androgenization affects the efficiency of β-adrenoceptor-mediated modulation of thymopoiesis. *J Neuroimmunol* (2011) 239:68–79. doi:10.1016/j.jneuroim.2011.08.020
36. Williams JM, Felten DL. Sympathetic innervation of murine thymus and spleen: a comparative histofluorescence study. *Anat Rec* (1981) 199:531–42. doi:10.1002/ar.1091990409
37. Leposavić G, Mićić M, Ugresić N, Bogojević M, Isaković K. Components of sympathetic innervation of the rat thymus during late fetal and postnatal development: histofluorescence and biochemical study. *Thymus* (1992) 19:77–87.
38. Vizi ES, Orso E, Osipenko ON, Hasko G, Elenkov IJ. Neurochemical, electrophysiological and immunocytochemical evidence for a noradrenergic link between the sympathetic nervous system and thymocytes. *Neuroscience* (1995) 68:1263–76. doi:10.1016/0306-4522(95)00215-5
39. Kurz B, Feindt J, von Gaudecker B, Kranz A, Loppnow H, Mentlein R. β-Adrenoceptor mediated effects in rat cultured thymic epithelial cells. *Br J Pharmacol* (1997) 120:1401–8. doi:10.1038/sj.bjp.0701045

40. Novotny GE, Sommerfeld H, Zirbes T. Thymic innervation in the rat: a light and electron microscopical study. *J Comp Neurol* (1990) 302:552–61. doi:10.1002/cne.903020311
41. Artico M, Cavallotti C, Cavallotti D. Adrenergic nerve fibres and mast cells: correlation in rat thymus. *Immunol Lett* (2002) 84:69–76. doi:10.1016/S0165-2478(02)00145-1
42. Leposavić G, Pilipović I, Radojević K. Immunomodulatory role of noradrenergic neural and non-neural system: role for β-adrenoceptor-mediated mechanisms in regulation of T-lymphocyte development. In: Endo M, Matsumoto N, editors. *Beta Blockers: New Research*. New York: Nova Science Publishers (2008). p. 141–82.
43. Singh U. Sympathetic innervation of fetal mouse thymus. *Eur J Immunol* (1984) 14:757–9. doi:10.1002/eji.1830140817
44. Pilipović I, Radojević K, Kosec D, Perišić Nanut M, Stojić-Vukanić Z, Arsenović-Ranin N, et al. Gonadal hormone dependent developmental plasticity of catecholamine:β2-adrenoceptor signaling complex in male rat thymus: putative implications for thymopoiesis. *J Neuroimmunol* (2013) 265:20–35. doi:10.1016/j.jneuroim.2013.09.021
45. Pilipović I, Vujnović I, Arsenović-Ranin N, Dimitrijević M, Kosec D, Stojić-Vukanić Z, et al. Peripubertal ovariectomy influences thymic adrenergic network plasticity in adult rats. *J Neuroimmunol* (2016) 297:103–16. doi:10.1016/j.jneuroim.2016.05.017
46. Bellinger DL, Felten SY, Felten DL. Maintenance of noradrenergic sympathetic innervation in the involuted thymus of the aged Fischer 344 rat. *Brain Behav Immun* (1988) 2:133–50. doi:10.1016/0889-1591(88)90014-1
47. Mićić M, Leposavić G, Ugrešić N. Relationships between monoaminergic and cholinergic innervation of the rat thymus during aging. *J Neuroimmunol* (1994) 49:205–12. doi:10.1016/0165-5728(94)90197-X
48. Madden KS, Bellinger DL, Felten SY, Snyder E, Maida ME, Felten DL. Alterations in sympathetic innervation of thymus and spleen in aged mice. *Mech Ageing Dev* (1998) 94:165–75. doi:10.1016/S0047-6374(96)01858-1
49. Cavallotti C, Artico M, Cavallotti D. Occurrence of adrenergic nerve fibers and of noradrenaline in thymus gland of juvenile and aged rats. *Immunol Lett* (1999) 70:53–62. doi:10.1016/S0165-2478(99)00127-3
50. Leposavić G, Pešić P, Stojić-Vukanić Z, Radojević K, Arsenović-Ranin N, Kosec D, et al. Age-associated plasticity of α1-adrenoceptor-mediated tuning of T-cell development. *Exp Gerontol* (2010) 45:918–35. doi:10.1016/j.exger.2010.08.011
51. Pilipović I, Vidić-Danković B, Perišić M, Radojević K, Čolić M, Todorović V, et al. Sexual dimorphism in the catecholamine-containing thymus microenvironment: a role for gonadal hormones. *J Neuroimmunol* (2008) 195:7–20. doi:10.1016/j.jneuroim.2007.12.006
52. Bergquist J, Tarkowski A, Ekman R, Ewing A. Discovery of endogenous catecholamines in lymphocytes and evidence for catecholamine regulation of lymphocyte function via an autocrine loop. *Proc Natl Acad Sci U S A* (1994) 91:12912–6. doi:10.1073/pnas.91.26.12912
53. Cosentino M, Bombelli R, Ferrari M, Marino F, Rasini M, Maestroni GJ, et al. HPLC-ED measurement of endogenous catecholamines in human immune cells and hematopoietic cell lines. *Life Sci* (2000) 68:283–95. doi:10.1016/S0024-3205(00)00937-1
54. Qiu YH, Peng YP, Jiang JM, Wang JJ. Expression of tyrosine hydroxylase in lymphocytes and effect of endogenous catecholamines on lymphocyte function. *Neuroimmunomodulation* (2004) 11:75–83. doi:10.1159/000075316
55. Radojević K, Rakin A, Pilipović I, Kosec D, Djikić J, Bufan B, et al. Effects of catecholamines on thymocyte apoptosis and proliferation depend on thymocyte microenvironment. *J Neuroimmunol* (2014) 272:16–28. doi:10.1016/j.jneuroim.2014.04.010
56. Takahama Y. Journey through the thymus: stromal guides for T-cell development and selection. *Nat Rev Immunol* (2006) 6:127–35. doi:10.1038/nri1781
57. Botham CA, Jones GV, Kendall MD. Immuno-characterisation of neuroendocrine cells of the rat thymus gland in vitro and in vivo. *Cell Tissue Res* (2001) 303:381–9. doi:10.1007/s004410000185
58. Jones GV, Botham CA, Clarke AG, Kendall MD. Immunoreactivity of neural crest-derived cells in thymic tissue developing under the rat kidney capsule. *Brain Behav Immun* (1998) 12:163–80. doi:10.1006/brbi.1998.0528
59. Anagnostou VK, Doussis-Anagnostopoulou I, Tiniakos DG, Karandrea D, Agapitos E, Karakitsos P, et al. Ontogeny of intrinsic innervation in the human thymus and spleen. *J Histochem Cytochem* (2007) 55:813–20. doi:10.1369/jhc.6A7168.2007
60. Leposavić G, Pilipović I, Radojević K, Pešić V, Perisić M, Kosec D. Catecholamines as immunomodulators: a role for adrenoceptor-mediated mechanisms in fine tuning of T-cell development. *Auton Neurosci* (2008) 144:1–12. doi:10.1016/j.autneu.2008.09.003
61. Huang MH, Bahl JJ, Wu Y, Hu F, Larson DF, Roeske WR, et al. Neuroendocrine properties of intrinsic cardiac adrenergic cells in fetal rat heart. *Am J Physiol Heart Circ Physiol* (2005) 288:497–503. doi:10.1152/ajpheart.00591.2004
62. Huang MH, Friend DS, Sunday ME, Singh K, Haley K, Austen KF, et al. An intrinsic adrenergic system in mammalian heart. *J Clin Invest* (1996) 98:1298–303. doi:10.1172/JCI118916
63. Miller LE, Grifka J, Schölmerich J, Straub RH. Norepinephrine from synovial tyrosine hydroxylase positive cells is a strong indicator of synovial inflammation in rheumatoid arthritis. *J Rheumatol* (2002) 29:427–35.
64. Kvetnansky R, Sabban EL, Palkovits M. Catecholaminergic systems in stress: structural and molecular genetic approaches. *Physiol Rev* (2009) 89:535–606. doi:10.1152/physrev.00042.2006
65. Tamura Y, Sano M, Nakamura H, Ito K, Sato Y, Shinmura K, et al. Neural crest-derived resident cardiac cells contribute to the restoration of adrenergic function of transplanted heart in rodent. *Cardiovasc Res* (2016) 109:350–7. doi:10.1093/cvr/cvv267
66. Capellino S, Weber K, Gelder M, Härle P, Straub RH. First appearance and location of catecholaminergic cells during experimental arthritis and elimination by chemical sympathectomy. *Arthritis Rheum* (2012) 64:1110–8. doi:10.1002/art.33431
67. Marchetti B, Morale MC, Pelletier G. The thymus gland as a major target for the central nervous system and the neuroendocrine system: neuroendocrine modulation of thymic β2-adrenergic receptor distribution as revealed by in vitro autoradiography. *Mol Cell Neurosci* (1990) 1:10–9. doi:10.1016/1044-7431(90)90037-5
68. Marchetti B, Morale MC, Pelletier G. Sympathetic nervous system control of thymus gland maturation: autoradiographic characterization and localization of the β2-adrenergic receptors in the rat thymus gland and presence of a sexual dimorphism during ontogenic development. *Prog Neuroendocr Immunol* (1990) 3:103–15.
69. Marchetti B, Morale MC, Paradis P, Bouvier M. Characterization, expression, and hormonal control of a thymic beta 2-adrenergic receptor. *Am J Physiol Endocrinol Metab* (1994) 267:718–31. doi:10.1152/ajpendo.1994.267.5.E718
70. Pesić V, Kosec D, Radojević K, Pilipović I, Perisić M, Vidić-Danković B, et al. Expression of alpha1-adrenoceptors on thymic cells and their role in fine tuning of thymopoiesis. *J Neuroimmunol* (2009) 214:55–66. doi:10.1016/j.jneuroim.2009.06.018
71. Fuchs BA, Albright JW, Albright JF. β-Adrenergic receptors on murine lymphocytes: density varies with cell maturity and lymphocyte subtype and is decreased after antigen administration. *Cell Immunol* (1988) 114:231–45. doi:10.1016/0008-8749(88)90318-8
72. Kavelaars A. Regulated expression of alpha-1 adrenergic receptors in the immune system. *Brain Behav Immun* (2002) 16:799–807. doi:10.1016/S0889-1591(02)00033-8
73. Leposavić G, Pilipović I, Perišić M. Cellular and nerve fibre catecholaminergic thymic network: steroid hormone dependent activity. *Physiol Res* (2011) 60:S71–S82.
74. Leposavić G, Pilipović I, Perišić M. Age-associated remodeling of neural and nonneural thymic catecholaminergic network affects thymopoietic productivity. *Neuroimmunomodulation* (2011) 18:290–308. doi:10.1159/000329499
75. Mackinnon PCB, Greenstein BD. Sexual differentiation of the brain. In: Faulkner F, Tanner JM, editors. *Human Growth. A Comprehensive Treatise. Vol. 2: Postnatal Growth; Neurobiology*. New York: Plenum Publishing Corporation (1986). p. 437–68.
76. Phoenix CH, Goy RW, Gerall AA, Young WC. Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. *Endocrinology* (1959) 65:369–82. doi:10.1210/endo-65-3-369
77. Gorski RA. Sexual differentiation of the hypothalamus. In: Mack H, Sherman AI, editors. *The Neuroendocrinology of Human Reproduction*. Springfield: CC Thomas Publisher (1971). p. 60–90.
78. Arai Y, Matsumoto A, Nishizuka M. Synaptogenesis and neuronal plasticity to gonadal steroids: implication for the development of sexual dimorphism in the neuroendocrin brain. In: Ganten D, Pfaff D, editors. *Current Topics in Neuroendocrinology*. Berlin: Springer (1986). p. 291–307.

79. Gorski RA, Gordon JH, Shryne JE, Southam AM. Evidence for a morphological sex difference within the medial preoptic area of the rat brain. *Brain Res* (1978) 148:333–46. doi:10.1016/0006-8993(78)90723-0
80. Tang FY, Bonfiglio TA, Tang LK. Effect of estrogen and progesterone on the development of endometrial hyperplasia in the Fischer rat. *Biol Reprod* (1984) 31:399–413. doi:10.1095/biolreprod31.2.399
81. Barraclough CA. Production of anovulatory, sterile rats by single injections of testosterone propionate. *Endocrinology* (1961) 68:62–7. doi:10.1210/endo-68-1-62
82. Thomas P, Pang Y, Dong J, Berg AH. Identification and characterization of membrane androgen receptors in the ZIP9 zinc transporter subfamily: II. Role of human ZIP9 in testosterone-induced prostate and breast cancer cell apoptosis. *Endocrinology* (2014) 155:4250–65. doi:10.1210/en.2014-1201
83. McCarthy MM. Estradiol and the developing brain. *Physiol Rev* (2008) 88:91–124. doi:10.1152/physrev.00010.2007
84. Filardo EJ, Thomas P. Minireview: G protein-coupled estrogen receptor-1, GPER-1: its mechanism of action and role in female reproductive cancer, renal and vascular physiology. *Endocrinology* (2012) 153:2953–62. doi:10.1210/en.2012-1061
85. Leposavić G, Pejčić-Karapetrovic B, Kosec D. Neonatal androgenization affects the intrathymic T-cell maturation in rats. *Neuroimmunomodulation* (2005) 12:117–30. doi:10.1159/000083584
86. Leposavić G, Perisić M, Kosec D, Arsenović-Ranin N, Radojević K, Stojić-Vukanić Z, et al. Neonatal testosterone imprinting affects thymus development and leads to phenotypic rejuvenation and masculinization of the peripheral blood T-cell compartment in adult female rats. *Brain Behav Immun* (2009) 23:294–304. doi:10.1016/j.bbi.2008.11.002
87. Hueber AO, Bernard AM, Battari CL, Marguet D, Massol P, Foa C, et al. Thymocytes in Thy-1-/- mice show augmented TCR signaling and impaired differentiation. *Curr Biol* (1997) 7:705–8. doi:10.1016/S0960-9822(06)00300-9
88. Shames RS. Gender differences in the development and function of the immune system. *J Adolesc Health* (2002) 30:59–70. doi:10.1016/S1054-139X(01)00382-2
89. Alonso-Solís R, Abreu P, López-Coviella I, Hernández G, Fajardo N, Hernández-Díaz F, et al. Gonadal steroid modulation of neuroendocrine transduction: a transynaptic view. *Cell Mol Neurobiol* (1996) 16:357–82. doi:10.1007/BF02088101
90. Simerly RB. Hormonal control of the development and regulation of tyrosine hydroxylase expression within a sexually dimorphic population of dopaminergic cells in the hypothalamus. *Brain Res Mol Brain Res* (1989) 6:297–310. doi:10.1016/0169-328X(89)90075-2
91. Plut C, Ribiere C, Giudicelli Y, Dausse JP. Gender differences in hypothalamic tyrosine hydroxylase and alpha(2)-adrenoceptor subtype gene expression in cafeteria diet-induced hypertension and consequences of neonatal androgenization. *J Pharmacol Exp Ther* (2002) 302:525–31. doi:10.1124/jpet.302.2.525
92. Jeong H, Kim MS, Kwon J, Kim KS, Seol W. Regulation of the transcriptional activity of the tyrosine hydroxylase gene by androgen receptor. *Neurosci Lett* (2006) 396:57–61. doi:10.1016/j.neulet.2005.11.011
93. Moawad AH, River LP, Kilpatrick SJ. The effect of estrogen and progesterone on beta-adrenergic receptor activity in rabbit lung tissue. *Am J Obstet Gynecol* (1982) 144:608–13. doi:10.1016/0002-9378(82)90235-6
94. Wu Q, Zhao Z, Sun H, Hao YL, Yan CD, Gu SL. Oestrogen changed cardiomyocyte contraction and beta-adrenoceptor expression in rat hearts subjected to ischaemia-reperfusion. *Exp Physiol* (2008) 93:1034–43. doi:10.1113/expphysiol.2007.041939
95. Arner P. Effects of testosterone on fat cell lipolysis. Species differences and possible role in polycystic ovarian syndrome. *Biochimie* (2005) 87:39–43. doi:10.1016/j.biochi.2004.11.012
96. Csaba G, Dobozy O, Inczefi-Gonda A, Szeberenyi S. Effect of the absence of neonatal testosterone imprinting on the activity of the microsomal enzyme system and on the dexamethasone binding of the thymus in adulthood. *Acta Physiol Hung* (1988) 71:421–7.
97. Csaba G, Inczefi-Gonda A. Effect of a single treatment (imprinting) with genistein or combined treatment with genistein+benzpyrene on the binding capacity of glucocorticoid and estrogen receptors of adult rats. *Hum Exp Toxicol* (2002) 21:231–4. doi:10.1191/0960327102ht242oa
98. Wajeman-Chao SA, Lancaster SA, Graf LH Jr, Chambers DA. Mechanism of catecholamine-mediated destabilization of messenger RNA encoding Thy-1 protein in T-lineage cells. *J Immunol* (1998) 161:4825–33.
99. Lajevic MD, Koduvayur SP, Caffrey V, Cohen RL, Chambers DA. Thy-1 mRNA destabilization by norepinephrine a 3′ UTR cAMP responsive decay element and involves RNA binding proteins. *Brain Behav Immun* (2010) 24:1078–88. doi:10.1016/j.bbi.2010.04.006
100. Kikuchi-Utsumi K, Kikuchi-Utsumi M, Cannon B, Nedergaard J. Differential regulation of the expression of alpha1-adrenergic receptor subtype genes in brown adipose tissue. *Biochem J* (1997) 322:417–24. doi:10.1042/bj3220417
101. Csaba G. The biological basis and clinical significance of hormonal imprinting, an epigenetic process *Clin Epigenetics* (2011) 2:187–96. doi:10.1007/s13148-011-0024-8
102. Da Silva JAP. Sex hormones and glucocorticoids: interactions with the immune system. *Ann N Y Acad Sci* (1999) 876:102–17. doi:10.1111/j.1749-6632.1999.tb07628.x
103. Schulz KM, Molenda-Figueira HA, Sisk CL. Back to the future: the organizational-activational hypothesis adapted to puberty and adolescence. *Horm Behav* (2009) 55:597–604. doi:10.1016/j.yhbeh.2009.03.010
104. Perišić M, Kosec D, Pilipović I, Radojević K, Pešić V, Rakin A, et al. Peripubertal ovariectomy provides long-term postponement of age-associated decline in thymic cellularity and T-cell output. *Acta Vet (Beogr)* (2009) 59:3–15. doi:10.2298/AVB0901003P
105. ThyagaRajan S, Madden KS, Teruya B, Stevens SY, Felten DL, Bellinger DL. Age-associated alterations in sympathetic noradrenergic innervation of primary and secondary lymphoid organs in female Fischer 344 rats. *J Neuroimmunol* (2011) 233:54–64. doi:10.1016/j.jneuroim.2010.11.012
106. Chávez-Genaro R, Crutcher K, Viettro L, Richeri A, Coirolo N, Burnstock G, et al. Differential effects of oestrogen on developing and mature uterine sympathetic nerves. *Cell Tissue Res* (2002) 308:61–73. doi:10.1007/s00441-002-0521-3
107. Thanky NR, Son JH, Herbison AE. Sex differences in the regulation of tyrosine hydroxylase gene transcription by estrogen in the locus coeruleus of TH9-LacZ transgenic mice. *Brain Res Mol Brain Res* (2002) 104:220–6. doi:10.1016/S0169-328X(02)00383-2
108. Nakashima A, Ota A, Sabban EL. Interactions between Egr1 and AP1 factors in regulation of tyrosine hydroxylase transcription. *Brain Res Mol Brain Res* (2003) 112:61–9. doi:10.1016/S0169-328X(03)00047-0
109. Fung BP, Yoon SO, Chikaraishi DM. Sequences that direct rat tyrosine hydroxylase gene expression. *J Neurochem* (1992) 58:2044–52. doi:10.1111/j.1471-4159.1992.tb10945.x
110. McDonnell DP, Norris JD. Connections and regulation of the human estrogen receptor. *Science* (2002) 296:1642–4. doi:10.1126/science.1071884
111. Serova LI, Maharjan S, Huang A, Sun D, Kaley G, Sabban EL. Response of tyrosine hydroxylase and GTP cyclohydrolase I gene expression to estrogen in brain catecholaminergic regions varies with mode of administration. *Brain Res* (2004) 1015:1–8. doi:10.1016/j.brainres.2004.04.002
112. Raab H, Pilgrim C, Reisert I. Effects of sex and estrogen on tyrosine hydroxylase mRNA in cultured embryonic rat mesencephalon. *Brain Res Mol Brain Res* (1995) 33:157–64. doi:10.1016/0169-328X(95)00125-C
113. Zhao H, Tian Z, Hao J, Chen B. Extragonadal aromatization increases with time after ovariectomy in rats. *Reprod Biol Endocrinol* (2005) 3:6. doi:10.1186/1477-7827-3-6

Conflict of Interest Statement: The authors declare that they have not any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 16 May 2018
doi: 10.3389/fendo.2018.00235

Regulatory and Mechanistic Actions of Glucocorticoids on T and Inflammatory Cells

*Ana C. Liberman[1†], Maia L. Budziñski[1†], Clara Sokn[1], Romina Paula Gobbini[1], Anja Steininger[1] and Eduardo Arzt[1,2]**

[1] Instituto de Investigación en Biomedicina de Buenos Aires (IBioBA) – CONICET – Partner Institute of the Max Planck Society, Buenos Aires, Argentina, [2] Departamento de Fisiología y Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires, Argentina

Glucocorticoids (GCs) play an important role in regulating the inflammatory and immune response and have been used since decades to treat various inflammatory and autoimmune disorders. Fine-tuning the glucocorticoid receptor (GR) activity is instrumental in the search for novel therapeutic strategies aimed to reduce pathological signaling and restoring homeostasis. Despite the primary anti-inflammatory actions of GCs, there are studies suggesting that under certain conditions GCs may also exert pro-inflammatory responses. For these reasons the understanding of the GR basic mechanisms of action on different immune cells in the periphery (e.g., macrophages, dendritic cells, neutrophils, and T cells) and in the brain (microglia) contexts, that we review in this chapter, is a continuous matter of interest and may reveal novel therapeutic targets for the treatment of immune and inflammatory response.

Keywords: glucocorticoids, inflammation, FKBP51, transactivation, transrepression

OPEN ACCESS

Edited by:
Ana Rosa Pérez, CONICET, Argentina

Reviewed by:
Tullio Florio, Università di Genova, Italy
Luciano David D'Attilio, CONICET Rosario, Argentina

****Correspondence:***
Eduardo Arzt
earzt@iboba-mpsp-conicet.gov.ar

[†]These authors have contributed equally to this work.

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: *12 January 2018*
Accepted: *25 April 2018*
Published: *16 May 2018*

Citation:
Liberman AC, Budziñski ML, Sokn C, Gobbini RP, Steininger A and Arzt E (2018) Regulatory and Mechanistic Actions of Glucocorticoids on T and Inflammatory Cells. Front. Endocrinol. 9:235. doi: 10.3389/fendo.2018.00235

INTRODUCTION

Living organisms must sustain a dynamic equilibrium in order to maintain homeostasis and survival which is constantly challenged by internal or external stressors. In order to appropriately cope with stressful stimuli, they have developed a highly conserved regulatory system. This neuroendocrine system consists mainly of the hypothalamic–pituitary–adrenal (HPA) axis and the autonomic nervous system. Glucocorticoids (GCs), are the end-product of the HPA axis, and play an important role in the maintenance of both resting and stress-related responses. If the stress response is dysregulated, homeostasis is altered and probably a wide range of adverse effects may appear on many vital physiological functions, such as growth, development, metabolism, reproduction, immune response, cognition, and behavior.

GCs act on almost all types of cells and in particular in the immune cells they have been shown to have powerful immunosuppressive and anti-inflammatory activities (1–5). As a result of their anti-inflammatory properties, GCs are widely used to help treat many different conditions, such as allergic, autoimmune, inflammatory, and hematological alterations. Interestingly, an accumulating body of evidence now strongly suggests that GCs can have both pro- and anti-inflammatory roles under specific conditions. The pro-inflammatory activity of GCs is most apparent in the central nervous system (CNS). These opposite effects work together in order to resolve cellular responses to inflammatory stimuli and also as a protective mechanism "priming" the immune cells to efficiently respond to the noxa or stressor and then restore homeostasis (6).

Upon peripheral or cerebral immune stimulation, the HPA axis is activated. When the immunogenic stress occurs in the brain, local inflammatory components activate the HPA axis. However, if the challenge takes place outside the brain, multiple pathways bring together stimulatory signals from the periphery to the HPA axis. Mounting evidence suggests that cytokine signals access to the brain through different pathways. These pathways mainly include: cytokines passing across the blood–brain barrier; by specific saturable transport molecules on the brain endothelium; activation of endothelial cells of brain capillaries that release second messengers within the brain parenchyma; transmission of cytokine signals *via* afferent nerve fibers and finally by peripherally activated monocytes that can enter into the brain (7–11). The induction of these different mechanisms modulates cytokine activity in the brain (12–14).

The accurate regulation of the HPA axis activity is critical, since GC imbalances can result in many different pathological conditions (13, 15). Long-term treatment with GCs may result in a plethora of harmful undesired side effects, such as diabetes, hypertension, growth retardation, dyslipidemia, osteoporosis, glaucoma, muscle atrophy, and is also related to many important behavioral alterations, among others (16, 17). Chronic exposure to GCs can also be associated with GC insensitivity, reducing the efficacy of the therapy (18). Also, alterations or deficits in the HPA axis response are tightly associated with a wide range of autoimmune and inflammatory diseases (19–24).

In this review, we will discuss the role of GCs on the immune and inflammatory cells in the periphery and also the physiological importance and mechanisms implicated in the apparent paradoxical functions of GCs in the brain in order to appropriately maintain a coordinated homeostatic response.

THE GLUCOCORTICOID RECEPTOR (GR)

As a small lipophilic hormone, GCs can rapidly diffuse into cells and exert their main actions. These actions are elicited by the binding of GCs to their intracellular receptor, the GR. The GR is a hormone-activated transcription factor (TF) that belongs to the superfamily of nuclear hormone receptors (25). GR is a modular protein composed of three distinct regions with different functions (**Figure 1A**). The N-terminal domain (NTD) contains a transactivation domain called activation function 1 (AF1) that is responsible for the transcriptional activation and is implicated in the association with coregulators and the basal transcription machinery. The DNA-binding domain (DBD) is composed of two zinc fingers that have been shown to be important for GR homodimerization and DNA-binding specificity. The hinge region, which separates the DBD from the ligand binding domain (LBD), is a flexible linker structure which is implicated in allowing proper DNA binding, dimerization, and nuclear translocation of the receptor (26). The C-terminal LBD, contains the ligand binding site and a second transactivation domain (AF2) regulated by hormone binding (27). The AF2 transactivation domain is important for the interaction with co-chaperones, coregulators, and other TFs (28). The LBD also encompasses a dimer interface which is critical for GR function and the binding of the heat shock protein (Hsp) 90 (29). The DBD and LBD both contain nuclear localization signals, which are important for GR nuclear translocation. The DBD also contains the nuclear export signal sequence (NES) which targets it for export from the cell nucleus to the cytoplasm through the nuclear pore complex.

Some degree in the heterogeneity in GR proteins may result from alternative splicing (30) (**Figure 1B**). The specificity and sensitivity of different target tissues to GCs has been reported to be related to GR isoforms (30). The GRα is the predominant isoform, and it is the one that transduces GCs signaling in the cell (31). There are other four additional splice variants identified: GRβ, GRγ, GR-A, and GR-P. GRβ differs from GRα in the carboxy terminal sequence, rendering GRβ non-responsive to GCs (32, 33), with no transcription of target genes. Therefore, GRβ can be described as a dominant negative inhibitor of GRα activity. GRβ does not bind GC agonists, however, it does bind to the GR antagonist RU-486 (34). GRβ can inhibit GRα transcriptional activity by different molecular mechanisms including competition for glucocorticoid response elements (GRE), interference with the activity of coregulators, and formation of inactive dimers (35, 36). In most tissues, GRβ is expressed at very low levels. However, abundant GRβ expression has been described especially in some inflammatory cells, such as lymphocytes and macrophages, and have been related to GCs resistance in diseases such as asthma (37), rheumatoid arthritis (38), ulcerative colitis (39), systemic lupus erythematosus (40), and acute lymphoblastic leukemia and chronic lymphocytic leukemia (41, 42). Considering that GRβ can inhibit GRα activity, the modulation of GRα/GRβ expression ratios may be an interesting approach to regulate GC sensitivity (42, 43). In addition, eight alternative translation initiation sites increase the repertory of GR proteins to almost 40 distinct isoforms of GR protein (44) (**Figure 1B**).

At the cellular level, GC availability is also modulated by enzymes of the 11β-hydroxysteroid dehydrogenase (11β-HSD) family, mainly 11β-HSD1 and 11β-HSD2 which regulate the conversion of active cortisol into inactive cortisone. 11β-HSD1 favors the conversion of cortisol from cortisone, increasing local GC activity (45). In contrast, 11β-HSD2 catalyzes cortisol to cortisone, thereby reducing GC availability. Thus, the balance in the expression of these two enzymes in a given tissue or cell, regulates GC-mediated responses. In addition, some studies show that inflammatory cytokine signaling modulates the relative expression of 11β-HSD genes, favoring 11β-HSD1 and inhibiting 11β-HSD2 (46, 47), adding another level of regulation of GC activity.

Another important level for fine-tuning the cellular response to GCs in different environmental situations is the modulation of GR activity by posttranslational modifications (PTMs). These PTMs include phosphorylation, acetylation, ubiquitination, and sumoylation, which may accurately regulate GR activity in response to diverse external stimuli (48) (**Figure 1A**). In particular, SUMO conjugation has been extensively described to modulate GR transcriptional activity (49–52). GR contains three consensus sumoylation sites. Two sumoylation sites located at the NTD have been demonstrated to be part of the synergy control (SC) motif sequence (50). The SC motifs consist of short regulatory sequences which are important for inhibiting the synergistic transactivation. SUMO conjugation to the two

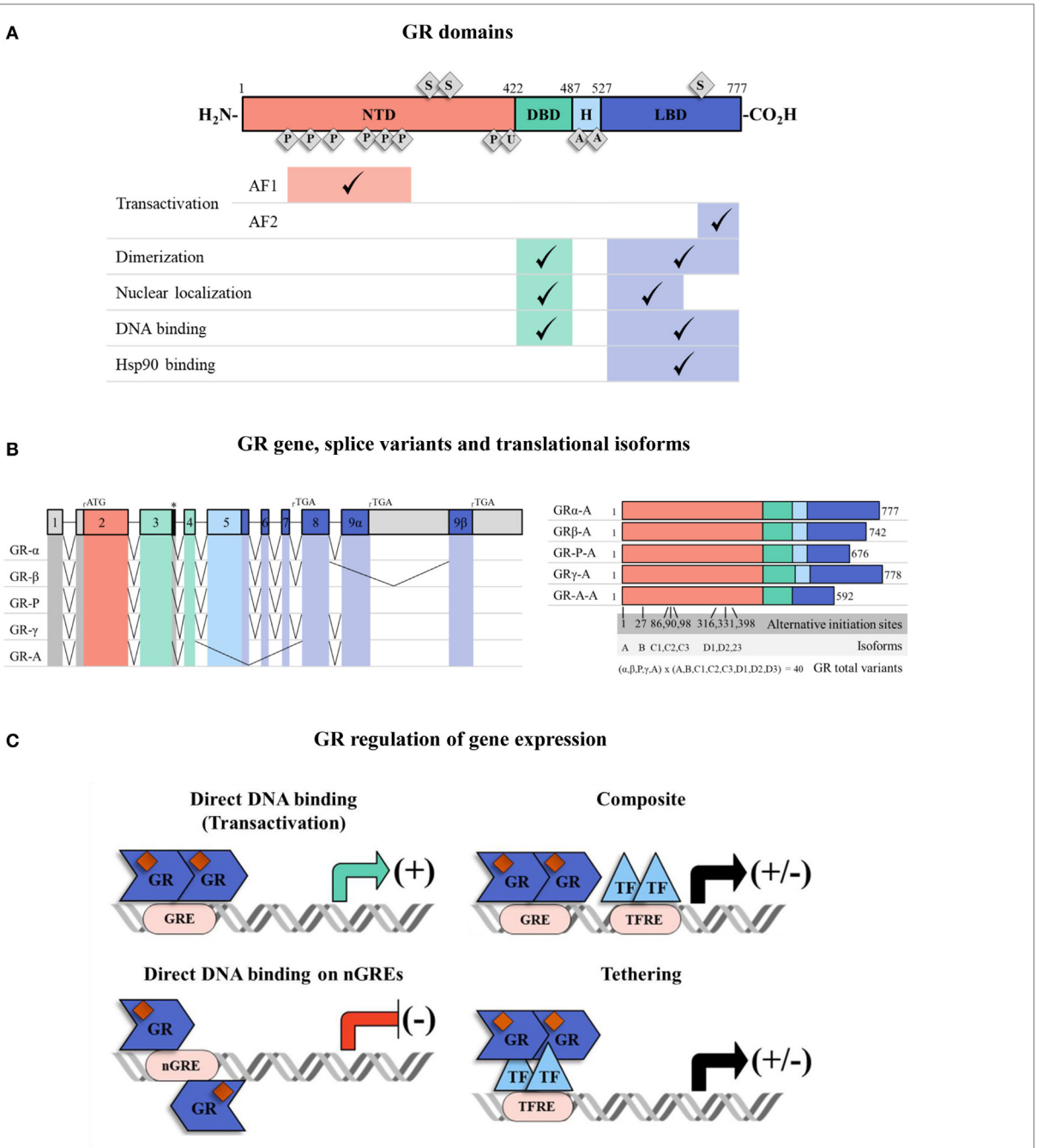

FIGURE 1 | The glucocorticoid receptor (GR) structure, isoforms, and mechanisms of transcriptional regulation. **(A)** Full human GRα protein has an N-terminal domain (NTD), a DNA-binding domain (DBD), a ligand binding domain (LBD) and a hinge region (H) between DBD and LBD. They have different associated functions, e.g., transactivation, dimerization, nuclear localization, DNA binding, and heat-shock protein 90 binding. The receptor can be post-translationally modified by phosphorylation (P), ubiquitination (U), acetylation (A) and sumoylation (S). Regions associated with transactivation (activation function 1 and 2: AF1 and AF2) are shown. **(B)** The GR has various isoforms which result from alternative splicing and multiple transcriptional start sites at exon 2. The colors indicate NTD (red, exon 2), DBD (green, exons 3–4), H (light blue, exon 5) and LBD (dark blue, exon 5–9). The 5′ and 3′-untranslated regions are colored in gray. There are five patterns of alternative splicing that result in GR isoforms α, β, P, γ, A. Each of them has eight translational variants (A, B, C1, C2, C3, D1, D2, D3) depending on the transcriptional start site ("*" denotes an alternative splice donor site in the intron between exons 3 and 4). **(C)** The GR, carrying GC ligand, translocates to the nucleus and regulates gene expression. GR can directly activate/inactivate gene expression by interacting with GREs/nGREs, it can bind to GREs and modulate gene transcription by interacting with neighboring DNA-bound transcription factors (TFs) (composite mechanism) and it can act by attaching itself to DNA-bound TFs (tethering mechanism). Abbreviations: TF, transcription factor; GRE, glucocorticoid response element; nGRE, negative glucocorticoid response element; TFRE, transcription factor response element.

NTD sumoylation sites is responsible for the functional effect of the SC motifs and thereby they inhibit GR activity (50, 53) (**Figure 1A**). It has also been demonstrated that in the presence of the sumoylation enhancer, RSUME (54), a SUMO peptide is conjugated to the third sumoylation site located in the LBD of the GR. Sumoylation in the LBD may be important for inducing GR-mediated transcriptional regulation during stress adaptation (55) (**Figure 1A**). A genome-wide analysis of GR sumoylation impact on gene expression, showed that genes differentially regulated by this PTM are mostly related to proliferation and apoptosis pathways and also strongly suggests that sumoylation can regulate genome-wide chromatin occupancy of the GR (56). Also, GR SUMO conjugation is influenced by other PTMs such as phosphorylation in order to fine-tune GR transcriptional activity in a target gene-specific manner (57). Important coregulators of the GR are also modified by SUMO conjugation, such as Hsp90, GRIP1, and also FKBP51, further regulating GR activity (58–62). Therefore, PTMs that impact on the GR but also on key molecules that fine-tune its activity, helps to understand the complexity of GR-mediated regulation of its target gene expression (2, 48).

GCs ANTI-INFLAMMATORY ACTIONS

The GR forms complex with chaperone molecules, such as Hsp90 and 70, and immunophilins, such as FKBP51, FKBP52, Cyp44, and PP5 (63). FKBP51 binds to the unbound GR and reduces GR activity mainly by reducing GR hormone binding and its nuclear translocation. Therefore, FKBP51 is considered as an inhibitor of GR transcriptional activity. Upon ligand binding, the GR exchanges FKBP51 for FKBP52, which is able to interact with the dynein motor protein, facilitating GR translocation to the nucleus (64). Interestingly, FKBP51 overexpression has been associated with GC resistance in autoimmune diseases. FKBP51 expression was found to be enhanced in sputum samples from patients with chronic obstructive pulmonary disease (65). Moreover, in a genome-wide profiling focused on the identification of epithelial gene markers of asthmatic patients and response to corticosteroids, GC treatment was found to induce FKBP51 expression, which in turn was associated with a poor response to corticosteroids, suggesting a role of FKBP51 in GC resistance (66, 67). Also, enhanced expression of FKBP51 has been found in bone marrow cells in patients with rheumatoid arthritis (68). Evidence also suggests that FKBP51 modulates NFκB-dependent gene expression, with possible implications for various inflammatory and immune pathways (69–73). Considering that GR is a key modulator of immune and inflammatory responses, FKBP51 dysregulation may provide the basis for a role of FKBP51 in these processes (66). Moreover, FKBP51 has recently been shown to be a target of SUMO conjugation and that sumoylation of FKBP51 is necessary for its association to Hsp90 and modulates FKBP51-mediated inhibition of GR activity in neuronal cells (58). In the brain, FKBP51 has been shown to be important for the development of psychiatric diseases and the response to antidepressant treatment, suggesting that regulation of FKBP51 activity might be an interesting approach for modulating GR outcome in the stress response and also in the inflammatory context (74–76).

Once in the nucleus, the activated GR can regulate gene expression by different mechanisms known as genomic effects (**Figure 1C**) (27). The genomic mechanism involves changes in the levels of specific genes: binding of GR to GREs in the promoters of its target genes and activation of transcription (transactivation); DNA binding of the GR with other TFs to "composite" elements which contain a GRE and an overlapping response element of another TF (binding can lead to gene activation or repression); or binding of the GR to a TF (e.g., NFκB; or AP1) by means of a "tethering" mechanism without contacting DNA, to influence the activity of the TF (this mechanism is considered to be the prevailing mechanism for transrepression) (2, 77, 78). Furthermore, GR-mediated transcriptional repression can be exerted *via* GR binding to a negative GRE (nGRE) (79). Binding to these nGRE prevent receptor dimerization through a strong negative cooperativity and alters the conformation of GR residues that are critical for transcriptional activation so that negative regulation is accomplished (80). A growing body of evidence shows that GC can also mediate non-genomic actions that do not require protein synthesis and are implicated in rapid cellular responses. For example, in the cytoplasm the activated GR can acutely interact with signaling pathways, such as PI3K, JNK, 14-3-3 proteins, and components of the T cell receptor signaling complex (81), modulating pro-inflammatory gene expression. In thymocytes, the activated GR can translocate to mitochondria and induce a rapid apoptotic response (82). In addition, membrane-bound GR on monocytes was reported to mediate non-genomic effects (82). On the other hand, binding of GCs to GR can modify the recruitment of different factors such as the multiprotein chaperone complex that participate in many signaling pathways, modifying secondary signaling cascades and, therefore, may further regulate the immune response (78, 83). GCs may also exert anti-inflammatory responses by direct negative interaction with components of the MAPK pathway, such as ERK, c-Jun NH2-terminal kinases (JNK), and p38 isoforms (p38) regulating their activity (84). Further studies are required to clarify the implications of non-genomic GC-mediated activity in the immune and inflammatory context.

It has been shown that several of the undesirable metabolic side effects associated with chronic GC treatment are mediated *via* transactivation. However the anti-inflammatory effects of GCs are mainly mediated *via* the transrepression elicited by a monomeric GR with the activity of TFs, such as NFκB and AP1 (1–3, 85). These TFs are involved in the activation of pro-inflammatory and immunoregulatory genes, such as inflammatory cytokines, cytokine receptors, adhesion molecules, and chemotactic proteins that play a key role for the coordination of the inflammatory response (1, 86–88). The first example of the transrepressive mechanism was the inhibitory interaction described between GR and AP1 (89), which results in the inhibition of IL2 expression (90). NFκB is present in almost all immune cells and regulates the expression of inflammatory cytokines. Thus, inhibition of NFκB activity is an important feature for GR-mediated anti-inflammatory activity (85, 91). It also inhibits NFAT-dependent IL2 transcription (92). The main mechanism of the GR action over these TFs is *via* transrepression: the activated GR acts by binding proximal to the NFκB or AP1-binding site and interacts

with these TFs inhibiting gene expression (93). The transrepression mechanism is not restricted to these TFs, but has expanded including among others, CREB, STAT, and T-bet (1–3, 94).

Alterations in chromatin structure have been reported to be important for regulating GC actions. The GR can differentially interact with proteins that have histone acetyltransferase (HAT) activity, but also with histone deacetylases and kinases that can influence the chromatin environment modifying chromatin accessibility and further regulating immune and inflammatory gene expression (3). In addition, chromatin accessibility has been reported to pre-determine GR binding patterns and, therefore, is critical for cell-specific outcome, providing new molecular basis for the tissue selectivity (95, 96). By all these different mechanisms, GCs regulate important functions, not only in the periphery but also in the brain.

Synthetic analogs of GC are often employed in the clinic in the therapy of allergic, inflammatory, and autoimmune disorders (97–99). It is generally accepted that GR-mediated transrepression holds the beneficial anti-inflammatory action, whereas their side effects are due mainly to the direct binding of GR to GREs as depicted before (98–100). However, transactivation is also necessary for the induction of several anti-inflammatory genes, such as MAP kinase phosphatase 1 (101), glucocorticoid-induced leucine zipper (102), and inhibitor kappa B-alpha (IκBα) (85). Therefore, the ideal GC analogs should be those that have high repressive activity against inflammatory mediators, but low transactivation activity, causing minimal side effects. Several steroidal and nonsteroidal ligands have been reported to have this dissociated function between transactivation and transrepressive mechanisms (97–99, 103). These compounds were shown to repress the activity of key inflammatory and immune TFs *in vivo* (104–107). However, GCs can induce gene expression not only by binding to GRE, but also in combination with other TFs and also by binding to promoter regions in a mechanism that does not involve GR dimerization or DNA interaction; therefore, unexpected secondary side effects might appear (78).

GCs may exert acute anti-inflammatory effects through the release of annexin-A1 (ANXA1) (108). Originally, this protein was suggested to have anti-inflammatory actions because it was described to inhibit phospholipase A2 (109). However, ANXA1 has been reported to regulate different cellular processes, such as migration, growth, differentiation, apoptosis, membrane fusion during exocytosis, lipid metabolism, and cytokine expression. Importantly, in the HPA axis, ANXA1 has been reported to play a critical role in the negative feedback exerted by GCs, therefore, affecting hypothalamic-releasing hormones secretion possibly *via* non-genomic mechanisms (110).

GCs ACTIVITY ON PERIPHERAL IMMUNE CELLS

GCs mediate immunosuppressive functions by acting on almost all types of immune cells (**Figure 2**). GCs can regulate the phenotype, survival, and functions of monocytes and macrophages which have crucial roles in tissue homeostasis and innate immunity. GCs exhibit anti-apoptotic effects promoting the survival of anti-inflammatory macrophages (111). The intrinsic molecular mechanism involves a prolonged induction of the extracellular signal-regulated kinase/MAPK (ERK/MAPK) pathway resulting in inhibition of caspase activities and expression of anti-apoptotic genes (111). GCs can also improve the phagocytic activity of these cells and stimulate the clearance of harmful elements, such as neutrophil clearance (112–114). GCs also suppress immunostimulatory functions of these cells and inhibit the release of various pro-inflammatory mediators, such as cytokines, chemokines, and reactive oxygen through different mechanisms (115, 116). Functional clustering of GC-regulated genes by human anti-inflammatory macrophages by microarray technology indicated induction of phagocytosis and motility as well as repression of adhesion, apoptosis, and oxidative burst (117, 118).

GCs can regulate the maturation, survival, and migration toward the lymph nodes and motility of dendritic cells (DCs), and also inhibit their immunogenic functions (**Figure 2**). GCs were shown to reduce the ability of DCs to stimulate T cells by inhibiting the upregulation of co-stimulatory molecules and cytokines, such as IL6, IL12, and TNFα and by inducing the tolerance-inducing transcription factor GILZ (119–125). The distinct actions exerted by GCs in immature and mature DCs are due to differential expression of GR translational isoforms (126).

GCs are important modulators of neutrophilia (**Figure 2**). Leukocyte extravasation is the movement of leukocytes out of the circulation and toward the site of tissue damage or infection. Rolling, adhesion, activation, and transmigration are necessary to arrive to the damaged tissue. GCs can modulate each of these steps. Rolling and adhesion is mediated by the interaction of the leukocyte integrins with the endothelial counterparts, which are inhibited by GCs (127–129). Also, GCs increase the number of circulating neutrophils in the blood stream by favoring their egress from the bone marrow and also inhibiting their migration to inflammatory sites by hindering the expression of adhesion molecules (32, 129, 130).

GCs exert distinct immunomodulatory actions on T cells (**Figure 2**). GCs decrease the number of circulating T cells by favoring their migration back to the bone marrow and secondary lymphoid tissues or through the induction of chemokine receptors, adhesion molecules, and matrix metalloproteinases (131, 132). The steroid hormone also favors T cells apoptosis. GC-induced apoptosis of T cells requires the dimerization of the GR (133) and is mediated *via* the induction of Puma and Bim expression (134–137). The relative expression of distinct GR isoforms increases the susceptibility of T cells to GC-induced cell death (138). Helper T (Th) cells are important players of the adaptive immunity (1). Upon antigen stimulation, naive Th cells can differentiate into different subsets: Th1, Th2, Th17, or regulatory T (Tregs) cells among others, each with specific effector functions. Th1 cells express the lineage-specific TF T-bet and STAT4 and release pro-inflammatory cytokines, such as IFNγ and IL2 (139). Th1 cells help in the activation of effector T cells, natural killer (NK) cells, and macrophages at the site of infection, promote effective immune responses against intracellular pathogens and are also implicated in autoimmune pathologies. Th2 lymphocytes selectively express the TF GATA3 and are characterized by the expression of IL5, IL4, IL10, and IL13 and are important for the

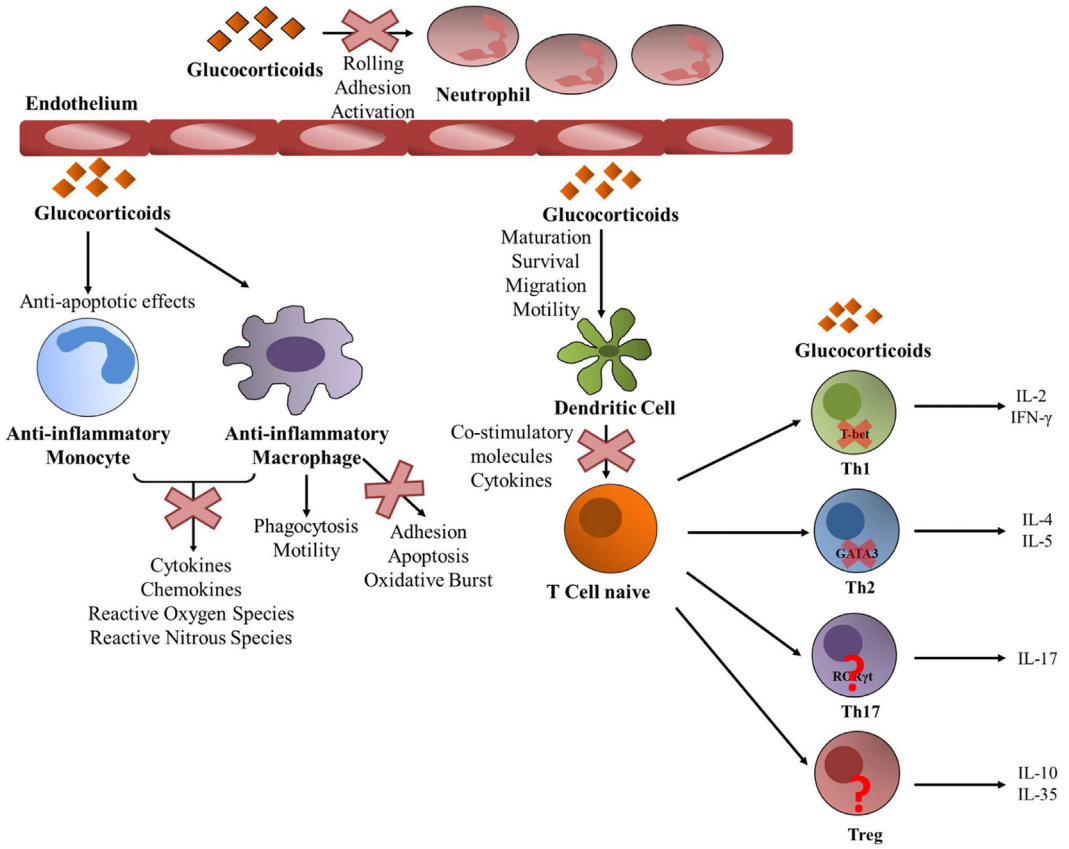

FIGURE 2 | Glucocorticoid (GC) activity on periphery immune cells. GCs act upon almost every immune cell type. GCs promote an anti-inflammatory state on both monocytes and macrophages. GCs prevent monocytes into entering apoptosis and inhibit the liberation of pro-inflammatory mediators by both types of cells. Particularly in macrophages, GCs promote phagocytosis and motility, while they inhibit adhesion, apoptosis and oxidative burst. They also act upon neutrophils function by inhibiting rolling, adhesion and activation. GCs act toward dendritic cells by promoting their maturation, survival, migration and motility, and at the same time GCs inhibit their ability to activate T cells by suppressing the production of pro-inflammatory molecules. A naïve helper T (Th) cell can differentiate into different Th lineages and GCs exert different actions. They act upon Th1 by decreasing T-bet transcriptional activity and suppressing the production of pro-inflammatory molecules such as IL-2 and IFNγ. They also suppress GATA3 activity in Th2 cells inhibiting the expression of IL-4 and IL-5. The action of GCs toward Th17 and regulatory T cells is not yet well understood.

proper eradication of extracellular pathogens (140). Also, Th2 cells activate B cells to produce antibodies and play a triggering role in the activation/recruitment of eosinophils and mast cells in allergic responses. IL17-producing Th17 cells selectively express RORγt and also RORα (141, 142). Th17 cells play an important role in autoimmune diseases and in host defense against infection. Treg cells mainly express the TF Foxp3 and inhibit effector T-cell differentiation and proliferation and suppress autoimmune and allergic responses (143). GCs inhibit the expression of many T cell cytokines (1) and can produce a shift from Th1-mediated cellular immunity to mediating humoral Th2 responses at physiological doses or chronic treatment (144). Upon acute treatment with GCs, they inhibit the synthesis of Th1 cytokines like IL2 and IFNγ and reduce STAT4 activity (145) and also reduce Th2 cytokines expression (146). The molecular mechanism by which GCs inhibit Th1 responses involves the reduction of T-bet transcriptional activity by the inhibitory interaction between GR and T-bet that results in diminished binding of T-bet to DNA (94) (**Figure 2**). Also GCs where shown to reduce mRNA and protein levels of T-bet (94). The activity of the Th2-specific TF GATA3 is also suppressed by GCs *via* two main mechanisms: first by GR-mediated inhibition of GATA3 translocation into the nucleus and second by the inhibition of GATA3 phosphorylation by GC-induced MKP1 expression (147, 148) (**Figure 2**). Furthermore, STAT6 activity also involved in Th2 differentiation is inhibited by GCs (149). How GCs modulate Th17-mediated responses has not been extensively studied, and the importance of Th17 modulation by GCs for the suppression of allergic or autoimmune diseases remains unclear (150). In rheumatoid arthritis, GC treatment diminished IL17 levels (151). In addition, in rat lymphocytes methylprednisolone inhibited IL17 expression due to the inhibition of RORγt expression (152) (**Figure 2**). However, several studies strongly suggest that GC resistance is associated with a pathogenic inflammatory Th17 phenotype that is refractory to GCs (150, 153, 154). Recently, a gene-expression profiling to characterize the steroid-resistant phenotype showed

that Th17 cells have restricted genome-wide responses to GCs and that they are refractory to GC inhibition at this level. In addition, Th17 cells were sensitive to suppression with the calcineurin inhibitor, cyclosporine A, suggesting that the clinical efficacy of cyclosporine A in the treatment of steroid resistance may be due to its selective inhibition of Th17 cells (155). Another interesting study has shown that Th17 cells are insensitive to GC-induced apoptosis and had high levels of BCL-2, knockdown of which sensitized Th17 cells to GC-induced cell death (156). Also, lung Th17 development in the murine severe asthma model was enhanced by GCs, supporting a role of Th17 cells in GC-refractory inflammatory conditions such as asthma (157).

In contrast to the inhibitory effect of GCs on pro-inflammatory effector T cells, it has been shown that Treg cells, which are key suppressors of T cell-dependent immune responses, are enhanced upon dexamethasone treatment by being more resistant to GC-induced cell death (158) (**Figure 2**). Also, GCs where shown to amplify IL2-dependent expansion of Treg cells and to enhance their capacity to reduce experimental autoimmune encephalomyelitis (EAE) in mice (159). In addition, GCs increase the percentage of Treg cells that express Foxp3 in patients with multiple sclerosis (160). *In vivo*, T cell-specific targeted GR deletion in pregnant animals undergoing EAE, resulted in a reduction of Treg population and a loss of pregnancy-induced protection, suggesting that steroid hormones can shift the immunological balance in favor of Tregs *via* differential engagement of the GR in T cells (161). However, others have found that GC treatment suppresses the expression of Foxp3 Tregs in an EAE model (162) and also in lungs of allergic mice (163).

In addition to their well-studied anti-inflammatory and immunosuppressive activity, an increasing body of evidence has revealed situations in which GCs have the opposite effect. This has been shown to depend on the dose, timing, duration of exposure, and cell population or tissue analyzed (164). The paradoxical pro-inflammatory role of GCs is mostly evident in the brain, where accumulating evidence show that GCs elicit different immune responses depending on the affected brain regions.

GCs ACTIONS IN THE BRAIN

There is a significant body of evidence indicating that GCs can suppress the innate immunity in the brain after a peripheral or cerebral challenge (23). In this way, in adrenalectomized mice, there is an induction in the levels of pro-inflammatory cytokines in the brain following LPS injection (165–168). Studies also demonstrated that GCs inhibit the release of pro-inflammatory mediators in microglial cells treated with LPS (169, 170). Experiments performed *in vivo* support these findings by revealing that dexamethasone causes a strong reduction in LPS induction of NFκB expression in the brain (171). In addition, COX inhibitors were demonstrated to increase the expression of pro-inflammatory genes in the brain during systemic inflammation by reducing the activation of the HPA axis and the release of GCs (172, 173). This same effect took place when the GR antagonist RU486 was administrated (172, 173). Also, systemic inflammation, through the increase in circulating GCs, has been reported to have the ability to prevent the cerebral innate immune response induced by intraparenchymal endotoxin injection (174). Mice treated with the GR antagonist RU486 before intracerebral LPS administration showed an increase in the pro-inflammatory response, which in turn induced neuronal death. These findings suggest that GCs are important for protecting the brain during innate immunity (175, 176). Interestingly, when mice lacking GR in microglia were challenged with an intracerebral administration of LPS, the activation of the toll-like receptor 4 signaling pathway induced cellular lesion, and also neuronal and axonal damage (177). In addition, microglial cell cultures have reduced motility and increased amoeboid morphology in the absence of GR expression. This study strongly suggests that microglial GR is the principal mediator preventing neuronal degeneration triggered by LPS and that it also contributes to the protection of other cell types (177), having an important role in promoting neuronal survival.

The majority of GC pro-inflammatory activity has been described in animal models of acute or chronic stress which occurred previous to peripheral or cerebral immune challenges. For instance, acute stressors were reported to induce the expression of pro-inflammatory cytokines in specific brain regions, such as the hippocampus, following LPS peripheral challenge (178–180). GCs were also found to upregulate microglial activation markers including the toll-like receptor 2 pro-inflammatory pathway (178, 181) (**Figure 3A**). It was also shown that chronic unpredictable stress was able to potentiate LPS-mediated activation of NFκB activity in the frontal cortex and hippocampus *via* GC production (182). Also, chronically stressed animals that were

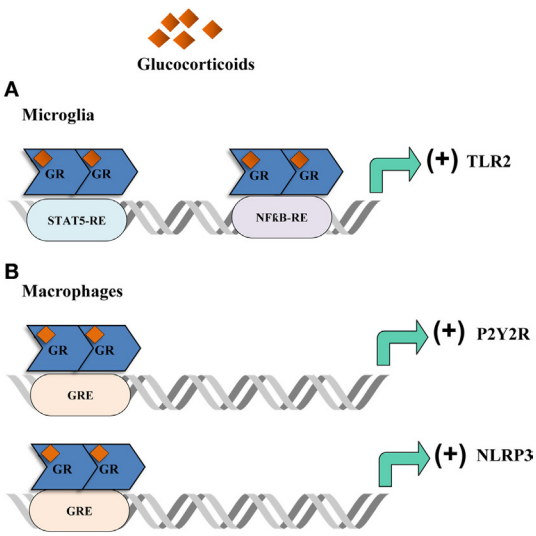

FIGURE 3 | Glucocorticoids (GCs) actions in the brain. Acute stressors promote an inflammatory phenotype in the brain. **(A)** In the microglia, GCs bind to the glucocorticoid receptor (GR) which then promotes the translation of the toll-like receptor 2 (TLR2) by interacting with STAT5 and NFκB response elements. TLR2 then exerts a pro-inflammatory response by promoting the production of inflammatory cytokines. **(B)** In macrophages, GCs promote the expression of the purinergic receptor P2Y2R which then produces IL-6 in response to ATP. Moreover, GCs enhance the expression of NLRP3 which in turn promotes the production of pro-inflammatory cytokines.

injected with LPS in the prefrontal cortex or the hippocampus, exhibited microglia activation, an increase in pro-inflammatory mediators and loss of astroglia and neurons. These effects were reduced with RU486 administration (183, 184). The prefrontal cortex is important in many brain functions and is a target for neurodegenerative diseases. It has been reported that in this brain region, TNFα expression and activation of MAPK signaling pathway is upregulated by chronic stress after intracortical LPS injection in a GR-dependent manner suggesting a synergistic effect between inflammation and stress. This fact could ultimately explain the relationship described between stress and some neurodegenerative pathologies (183, 184). In order to investigate if stress-induced GCs is responsible for the response of brain immune cells to pro-inflammatory stimuli, animals were acutely stressed and 24 h later hippocampal microglia were challenged with LPS *ex vivo*. Treatment *in vivo* with RU486 and adrenalectomized inhibited the microglial pro-inflammatory response, indicating that stress-induced GCs are able to sensitize the microglial pro-inflammatory function (185, 186). Therefore, stress may act "priming" central innate immunity to a subsequent immune challenge by making the neuroimmune context more responsive to inflammation, also favoring GC insensitivity or reducing the HPA response (187). In addition, acute restraint stress, inescapable tail shock and other stressors induce many inflammatory mediators, reduce immunoregulatory proteins and trigger microglia activation and proliferation (188–193). In addition, GCs have been reported to increase the expression of the purinergic receptor P2Y2R (**Figure 3B**) which promotes the secretion of inflammatory mediators in response to ATP (194). Recent data also indicate that GCs induce the expression of NLRP3 (NLRP3: nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing 3) in macrophages, which is a critical component of the inflammasome (**Figure 3B**). The GC-dependent induction of NLRP3 sensitizes the cells to extracellular ATP and significantly enhances the ATP-mediated release of pro-inflammatory molecules. This effect was specific for GCs and dependent on the GR and suggests that GCs sensitize the initial inflammatory response in the context of acute cellular damage or death (32). In addition, GCs and TNFα were shown to coregulate immune gene expression when combined (195). These results suggest that the final outcome of GCs pro- or anti-inflammatory activity depends on the activation state and signaling context. GCs are also able to modulate the inflammatory response to LPS in different ways according to the brain region (180, 182). For example, GR activation during chronic stress increases LPS-induced NFκB activation and TNFα, IL1β, and iNOS expression in the hippocampus and frontal cortex, but exhibits contrary effects in the hypothalamus (182). It is important to keep in mind that a pro-inflammatory context does not necessarily mean that damage will take place. Timing is a key parameter that will determine the final outcome of the inflammatory response. While exaggerated inflammation can favor neuronal dysfunction and cell death, pro-inflammatory mediators may at first induce the removal of the pathogen, the recruitment of immune cells and initiate tissue remodeling in order to appropriately cope with the pathogen and therefore, restoring homeostasis.

CONCLUSION

GCs are widely used in the clinic to control not only peripheral, but also CNS inflammatory response. However, the prolonged administration of this steroid hormone is often ineffective and can even worsen the outcome of the disease. Considering the known undesirable metabolic side effect, the induction of pro-inflammatory responses and the existence of GC resistance, GCs should be used carefully. Future research should be focused not only in understanding the molecular basis of GCs side effects and resistance, but also in dissecting how GCs induce pro-inflammatory responses in order to avoid serious detrimental consequences, particularly in the brain. In the future, a combination of different therapeutic approaches may lead to a more effective treatment and may help to lower the doses or duration of GC treatment thus minimizing the risk of toxicity and drug resistance (196). Finally, taking into account inter-individual differences in patient responsiveness to GC treatment, where different molecular mechanisms might be implicated, future directions should be in support of a customized and personalized treatment to meet individual patient needs.

AUTHOR CONTRIBUTIONS

AL: wrote, discussed, and corrected the manuscript. MB: discussed and corrected the manuscript. CS: discussed and corrected the manuscript, performed the figures. RG: discussed and corrected the manuscript, performed the figures. AS: corrected the manuscript. EA: discussed and corrected the manuscript.

FUNDING

This work was supported by grants from the Max Planck Society, Germany; the University of Buenos Aires; CONICET; the Agencia Nacional de Promoción Científica y Tecnológica, Argentina; and FOCEM-Mercosur (COF 03/11).

REFERENCES

1. Liberman AC, Druker J, Perone MJ, Arzt E. Glucocorticoids in the regulation of transcription factors that control cytokine synthesis. *Cytokine Growth Factor Rev* (2007) 18:45–56. doi:10.1016/j.cytogfr.2007.01.005
2. Petta I, Dejager L, Ballegeer M, Lievens S, Tavernier J, De Bosscher K, et al. The interactome of the glucocorticoid receptor and its influence on the actions of glucocorticoids in combatting inflammatory and infectious diseases. *Microbiol Mol Biol Rev* (2016) 80:495–522. doi:10.1128/MMBR.00064-15
3. Ratman D, Vanden Berghe W, Dejager L, Libert C, Tavernier J, Beck IM, et al. How glucocorticoid receptors modulate the activity of other transcription factors: a scope beyond tethering. *Mol Cell Endocrinol* (2013) 380:41–54. doi:10.1016/j.mce.2012.12.014
4. Refojo D, Liberman AC, Holsboer F, Arzt E. Transcription factor-mediated molecular mechanisms involved in the functional cross-talk between

cytokines and glucocorticoids. *Immunol Cell Biol* (2001) 79:385–94. doi:10.1046/j.1440-1711.2001.01023.x
5. Stahn C, Buttgereit F. Genomic and nongenomic effects of glucocorticoids. *Nat Clin Pract Rheumatol* (2008) 4:525–33. doi:10.1038/ncprheum0898
6. Busillo JM, Cidlowski JA. The five Rs of glucocorticoid action during inflammation: ready, reinforce, repress, resolve, and restore. *Trends Endocrinol Metab* (2013) 24:109–19. doi:10.1016/j.tem.2012.11.005
7. D'Mello C, Le T, Swain MG. Cerebral microglia recruit monocytes into the brain in response to tumor necrosis factoralpha signaling during peripheral organ inflammation. *J Neurosci* (2009) 29:2089–102. doi:10.1523/JNEUROSCI.3567-08.2009
8. Konsman JP, Parnet P, Dantzer R. Cytokine-induced sickness behaviour: mechanisms and implications. *Trends Neurosci* (2002) 25:154–9. doi:10.1016/S0166-2236(00)02088-9
9. Plotkin SR, Banks WA, Kastin AJ. Comparison of saturable transport and extracellular pathways in the passage of interleukin-1 alpha across the blood-brain barrier. *J Neuroimmunol* (1996) 67:41–7. doi:10.1016/S0165-5728(96)00036-7
10. Rivest S, Lacroix S, Vallières L, Nadeau S, Zhang J, Laflamme N. How the blood talks to the brain parenchyma and the paraventricular nucleus of the hypothalamus during systemic inflammatory and infectious stimuli. *Proc Soc Exp Biol Med* (2000) 223:22–38. doi:10.1111/j.1525-1373.2000.22304.x
11. Watkins LR, Goehler LE, Relton JK, Tartaglia N, Silbert L, Martin D, et al. Blockade of interleukin-1 induced hyperthermia by subdiaphragmatic vagotomy: evidence for vagal mediation of immune-brain communication. *Neurosci Lett* (1995) 183:27–31. doi:10.1016/0304-3940(94)11105-R
12. Capuron L, Miller AH. Immune system to brain signaling: neuropsychopharmacological implications. *Pharmacol Ther* (2011) 130:226–38. doi:10.1016/j.pharmthera.2011.01.014
13. Pereda MP, Lohrer P, Kovalovsky D, Perez Castro C, Goldberg V, Losa M, et al. Interleukin-6 is inhibited by glucocorticoids and stimulates ACTH secretion and POMC expression in human corticotroph pituitary adenomas. *Exp Clin Endocrinol Diabetes* (2000) 108:202–7. doi:10.1055/s-2000-7887
14. Turrin NP, Rivest S. Unraveling the molecular details involved in the intimate link between the immune and neuroendocrine systems. *Exp Biol Med (Maywood)* (2004) 229:996–1006. doi:10.1177/153537020422901003
15. Cruz-Topete D, Cidlowski JA. One hormone, two actions: anti- and pro-inflammatory effects of glucocorticoids. *Neuroimmunomodulation* (2015) 22:20–32. doi:10.1159/000362724
16. Howell MP, Muglia LJ. Effects of genetically altered brain glucocorticoid receptor action on behavior and adrenal axis regulation in mice. *Front Neuroendocrinol* (2006) 27:275–84. doi:10.1016/j.yfrne.2006.05.001
17. Kleiman A, Tuckermann JP. Glucocorticoid receptor action in beneficial and side effects of steroid therapy: lessons from conditional knockout mice. *Mol Cell Endocrinol* (2007) 275:98–108. doi:10.1016/j.mce.2007.05.009
18. Barnes PJ, Adcock IM. Glucocorticoid resistance in inflammatory diseases. *Lancet* (2009) 373:1905–17. doi:10.1016/S0140-6736(09)60326-3
19. Bertini R, Bianchi M, Ghezzi P. Adrenalectomy sensitizes mice to the lethal effects of interleukin 1 and tumor necrosis factor. *J Exp Med* (1988) 167:1708–12. doi:10.1084/jem.167.5.1708
20. Edwards CK III, Yunger LM, Lorence RM, Dantzer R, Kelley KW. The pituitary gland is required for protection against lethal effects of *Salmonella typhimurium*. *Proc Natl Acad Sci U S A* (1991) 88:2274–7. doi:10.1073/pnas.88.6.2274
21. MacPhee IA, Antoni FA, Mason DW. Spontaneous recovery of rats from experimental allergic encephalomyelitis is dependent on regulation of the immune system by endogenous adrenal corticosteroids. *J Exp Med* (1989) 169:431–45. doi:10.1084/jem.169.2.431
22. Ramachandra RN, Sehon AH, Berczi I. Neuro-hormonal host defence in endotoxin shock. *Brain Behav Immun* (1992) 6:157–69. doi:10.1016/0889-1591(92)90015-G
23. Refojo D, Liberman AC, Giacomini D, Carbia Nagashima A, Graciarena M, Echenique C, et al. Integrating systemic information at the molecular level: cross-talk between steroid receptors and cytokine signaling on different target cells. *Ann N Y Acad Sci* (2003) 992:196–204. doi:10.1111/j.1749-6632.2003.tb03150.x
24. Ruzek MC, Pearce BD, Miller AH, Biron CA. Endogenous glucocorticoids protect against cytokine-mediated lethality during viral infection. *J Immunol* (1999) 162:3527–33.
25. Evans RM. The steroid and thyroid hormone receptor superfamily. *Science* (1988) 240:889–95. doi:10.1126/science.3283939
26. Kumar R, Thompson EB. Gene regulation by the glucocorticoid receptor: structure: function relationship. *J Steroid Biochem Mol Biol* (2005) 94:383–94. doi:10.1016/j.jsbmb.2004.12.046
27. Beck IM, Vanden Berghe W, Vermeulen L, Yamamoto KR, Haegeman G, De Bosscher K. Crosstalk in inflammation: the interplay of glucocorticoid receptor-based mechanisms and kinases and phosphatases. *Endocr Rev* (2009) 30:830–82. doi:10.1210/er.2009-0013
28. Glass CK, Rose DW, Rosenfeld MG. Nuclear receptor coactivators. *Curr Opin Cell Biol* (1997) 9:222–32. doi:10.1016/S0955-0674(97)80066-X
29. Bledsoe RK, Montana VG, Stanley TB, Delves CJ, Apolito CJ, McKee DD, et al. Crystal structure of the glucocorticoid receptor ligand binding domain reveals a novel mode of receptor dimerization and coactivator recognition. *Cell* (2002) 110:93–105. doi:10.1016/S0092-8674(02)00817-6
30. Cain DW, Cidlowski JA. Specificity and sensitivity of glucocorticoid signaling in health and disease. *Best Pract Res Clin Endocrinol Metab* (2015) 29:545–56. doi:10.1016/j.beem.2015.04.007
31. Oakley RH, Cidlowski JA. Cellular processing of the glucocorticoid receptor gene and protein: new mechanisms for generating tissue-specific actions of glucocorticoids. *J Biol Chem* (2011) 286:3177–84. doi:10.1074/jbc.R110.179325
32. Busillo JM, Azzam KM, Cidlowski JA. Glucocorticoids sensitize the innate immune system through regulation of the NLRP3 inflammasome. *J Biol Chem* (2011) 286:38703–13. doi:10.1074/jbc.M111.275370
33. Ligr M, Li Y, Logan SK, Taneja S, Melamed J, Lepor H, et al. Mifepristone inhibits GRbeta coupled prostate cancer cell proliferation. *J Urol* (2012) 188:981–8. doi:10.1016/j.juro.2012.04.102
34. Lewis-Tuffin LJ, Jewell CM, Bienstock RJ, Collins JB, Cidlowski JA. Human glucocorticoid receptor beta binds RU-486 and is transcriptionally active. *Mol Cell Biol* (2007) 27:2266–82. doi:10.1128/MCB.01439-06
35. Charmandari E, Chrousos GP, Ichijo T, Bhattacharyya N, Vottero A, Souvatzoglou E, et al. The human glucocorticoid receptor (hGR) beta isoform suppresses the transcriptional activity of hGRalpha by interfering with formation of active coactivator complexes. *Mol Endocrinol* (2005) 19:52–64. doi:10.1210/me.2004-0112
36. Oakley RH, Jewell CM, Yudt MR, Bofetiado DM, Cidlowski JA. The dominant negative activity of the human glucocorticoid receptor beta isoform. Specificity and mechanisms of action. *J Biol Chem* (1999) 274:27857–66. doi:10.1074/jbc.274.39.27857
37. Sousa AR, Lane SJ, Cidlowski JA, Staynov DZ, Lee TH. Glucocorticoid resistance in asthma is associated with elevated in vivo expression of the glucocorticoid receptor beta-isoform. *J Allergy Clin Immunol* (2000) 105:943–50. doi:10.1067/mai.2000.106486
38. Chikanza IC. Mechanisms of corticosteroid resistance in rheumatoid arthritis: a putative role for the corticosteroid receptor beta isoform. *Ann N Y Acad Sci* (2002) 966:39–48. doi:10.1111/j.1749-6632.2002.tb04200.x
39. Honda M, Orii F, Ayabe T, Imai S, Ashida T, Obara T, et al. Expression of glucocorticoid receptor beta in lymphocytes of patients with glucocorticoid-resistant ulcerative colitis. *Gastroenterology* (2000) 118:859–66. doi:10.1016/S0016-5085(00)70172-7
40. Piotrowski P, Burzynski M, Lianeri M, Mostowska M, Wudarski M, Chwalinska-Sadowska H, et al. Glucocorticoid receptor beta splice variant expression in patients with high and low activity of systemic lupus erythematosus. *Folia Histochem Cytobiol* (2007) 45:339–42.
41. Koga Y, Matsuzaki A, Suminoe A, Hattori H, Kanemitsu S, Hara T. Differential mRNA expression of glucocorticoid receptor alpha and beta is associated with glucocorticoid sensitivity of acute lymphoblastic leukemia in children. *Pediatr Blood Cancer* (2005) 45:121–7. doi:10.1002/pbc.20308
42. Shahidi H, Vottero A, Stratakis CA, Taymans SE, Karl M, Longui CA, et al. Imbalanced expression of the glucocorticoid receptor isoforms in cultured lymphocytes from a patient with systemic glucocorticoid resistance and chronic lymphocytic leukemia. *Biochem Biophys Res Commun* (1999) 254:559–65. doi:10.1006/bbrc.1998.9980
43. Lu NZ, Collins JB, Grissom SF, Cidlowski JA. Selective regulation of bone cell apoptosis by translational isoforms of the glucocorticoid receptor. *Mol Cell Biol* (2007) 27:7143–60. doi:10.1128/MCB.00253-07
44. Cain DW, Cidlowski JA. Immune regulation by glucocorticoids. *Nat Rev Immunol* (2017) 17:233–47. doi:10.1038/nri.2017.1

45. Cooper MS, Stewart PM. 11Beta-hydroxysteroid dehydrogenase type 1 and its role in the hypothalamus-pituitary-adrenal axis, metabolic syndrome, and inflammation. *J Clin Endocrinol Metab* (2009) 94:4645–54. doi:10.1210/jc.2009-1412
46. Stegk JP, Ebert B, Martin HJ, Maser E. Expression profiles of human 11beta-hydroxysteroid dehydrogenases type 1 and type 2 in inflammatory bowel diseases. *Mol Cell Endocrinol* (2009) 301:104–8. doi:10.1016/j.mce.2008.10.030
47. Zbánková S, Bryndová J, Leden P, Kment M, Svec A, Pácha J. 11Beta-hydroxysteroid dehydrogenase 1 and 2 expression in colon from patients with ulcerative colitis. *J Gastroenterol Hepatol* (2007) 22:1019–23. doi:10.1111/j.1440-1746.2006.04529.x
48. Liberman AC, Antunica-Noguerol M, Arzt E. Modulation of the glucocorticoid receptor activity by post-translational modifications. *Nucl Recept Res* (2014) 1:1–15. doi:10.11131/2014/101086
49. Golebiowski F, Matic I, Tatham MH, Cole C, Yin Y, Nakamura A, et al. System-wide changes to SUMO modifications in response to heat shock. *Sci Signal* (2009) 2:ra24. doi:10.1126/scisignal.2000282
50. Holmstrom S, Van Antwerp ME, Iñiguez-Lluhi JA. Direct and distinguishable inhibitory roles for SUMO isoforms in the control of transcriptional synergy. *Proc Natl Acad Sci U S A* (2003) 100:15758–63. doi:10.1073/pnas.2136933100
51. Le Drean Y, Mincheneau N, Le Goff P, Michel D. Potentiation of glucocorticoid receptor transcriptional activity by sumoylation. *Endocrinology* (2002) 143:3482–9. doi:10.1210/en.2002-220135
52. Tian S, Poukka H, Palvimo JJ, Jänne OA. Small ubiquitin-related modifier-1 (SUMO-1) modification of the glucocorticoid receptor. *Biochem J* (2002) 367:907–11. doi:10.1042/bj20021085
53. Iñiguez-Lluhí JA, Pearce D. A common motif within the negative regulatory regions of multiple factors inhibits their transcriptional synergy. *Mol Cell Biol* (2000) 20:6040–50. doi:10.1128/MCB.20.16.6040-6050.2000
54. Carbia-Nagashima A, Gerez J, Perez-Castro C, Paez-Pereda M, Silberstein S, Stalla GK, et al. RSUME, a small RWD-containing protein, enhances SUMO conjugation and stabilizes HIF-1alpha during hypoxia. *Cell* (2007) 131:309–23. doi:10.1016/j.cell.2007.07.044
55. Druker J, Liberman AC, Antunica-Noguerol M, Gerez J, Paez-Pereda M, Rein T, et al. RSUME enhances glucocorticoid receptor SUMOylation and transcriptional activity. *Mol Cell Biol* (2013) 33:2116–27. doi:10.1128/MCB.01470-12
56. Paakinaho V, Kaikkonen S, Makkonen H, Benes V, Palvimo JJ. SUMOylation regulates the chromatin occupancy and anti-proliferative gene programs of glucocorticoid receptor. *Nucleic Acids Res* (2014) 42:1575–92. doi:10.1093/nar/gkt1033
57. Davies L, Karthikeyan N, Lynch JT, Sial EA, Gkourtsa A, Demonacos C, et al. Cross talk of signaling pathways in the regulation of the glucocorticoid receptor function. *Mol Endocrinol* (2008) 22:1331–44. doi:10.1210/me.2007-0360
58. Antunica-Noguerol M, Budziñski ML, Druker J, Gassen NC, Sokn MC, Senin S, et al. The activity of the glucocorticoid receptor is regulated by SUMO conjugation to FKBP51. *Cell Death Differ* (2016) 23:1579–91. doi:10.1038/cdd.2016.44
59. Kotaja N, Karvonen U, Jänne OA, Palvimo JJ. The nuclear receptor interaction domain of GRIP1 is modulated by covalent attachment of SUMO-1. *J Biol Chem* (2002) 277:30283–8. doi:10.1074/jbc.M204768200
60. Panse VG, Hardeland U, Werner T, Kuster B, Hurt E. A proteome-wide approach identifies sumoylated substrate proteins in yeast. *J Biol Chem* (2004) 279:41346–51. doi:10.1074/jbc.M407950200
61. Pountney DL, Raftery MJ, Chegini F, Blumbergs PC, Gai WP. NSF, Unc-18-1, dynamin-1 and HSP90 are inclusion body components in neuronal intranuclear inclusion disease identified by anti-SUMO-1-immunocapture. *Acta Neuropathol* (2008) 116:603–14. doi:10.1007/s00401-008-0437-4
62. Zhou W, Ryan JJ, Zhou H. Global analyses of sumoylated proteins in *Saccharomyces cerevisiae*. Induction of protein sumoylation by cellular stresses. *J Biol Chem* (2004) 279:32262–8. doi:10.1074/jbc.M404173200
63. Pratt WB, Toft DO. Regulation of signaling protein function and trafficking by the hsp90/hsp70-based chaperone machinery. *Exp Biol Med (Maywood)* (2003) 228:111–33. doi:10.1177/153537020322800201
64. Schülke JP, Wochnik GM, Lang-Rollin I, Gassen NC, Knapp RT, Berning B, et al. Differential impact of tetratricopeptide repeat proteins on the steroid hormone receptors. *PLoS One* (2010) 5:e11717. doi:10.1371/journal.pone.0011717
65. Holownia A, Mroz RM, Kolodziejczyk A, Chyczewska E, Braszko JJ. Increased FKBP51 in induced sputum cells of chronic obstructive pulmonary disease patients after therapy. *Eur J Med Res* (2009) 14(Suppl 4):108–11.
66. Storer CL, Dickey CA, Galigniana MD, Rein T, Cox MB. FKBP51 and FKBP52 in signaling and disease. *Trends Endocrinol Metab* (2011) 22:481–90. doi:10.1016/j.tem.2011.08.001
67. Woodruff PG, Boushey HA, Dolganov GM, Barker CS, Yang YH, Donnelly S, et al. Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. *Proc Natl Acad Sci U S A* (2007) 104:15858–63. doi:10.1073/pnas.0707413104
68. Matsushita R, Hashimoto A, Tomita T, Yoshitawa H, Tanaka S, Endo H, et al. Enhanced expression of mRNA for FK506-binding protein 5 in bone marrow CD34 positive cells in patients with rheumatoid arthritis. *Clin Exp Rheumatol* (2010) 28:87–90.
69. Baker RG, Hayden MS, Ghosh S. NF-kappaB, inflammation, and metabolic disease. *Cell Metab* (2011) 13:11–22. doi:10.1016/j.cmet.2010.12.008
70. Erlejman AG, De Leo SA, Mazaira GI, Molinari AM, Camisay MF, Fontana V, et al. NF-kappaB transcriptional activity is modulated by FK506-binding proteins FKBP51 and FKBP52: a role for peptidyl-prolyl isomerase activity. *J Biol Chem* (2014) 289:26263–76. doi:10.1074/jbc.M114.582882
71. Hayden MS, Ghosh S. NF-kappaB in immunobiology. *Cell Res* (2011) 21:223–44. doi:10.1038/cr.2011.13
72. Nakamura N, Shimaoka Y, Tougan T, Onda H, Okuzaki D, Zhao H, et al. Isolation and expression profiling of genes upregulated in bone marrow-derived mononuclear cells of rheumatoid arthritis patients. *DNA Res* (2006) 13:169–83. doi:10.1093/dnares/dsl006
73. Park J, Kim M, Na G, Jeon I, Kwon YK, Kim JH, et al. Glucocorticoids modulate NF-kappaB-dependent gene expression by up-regulating FKBP51 expression in Newcastle disease virus-infected chickens. *Mol Cell Endocrinol* (2007) 278:7–17. doi:10.1016/j.mce.2007.08.002
74. Binder EB, Salyakina D, Lichtner P, Wochnik GM, Ising M, Pütz B, et al. Polymorphisms in FKBP5 are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. *Nat Genet* (2004) 36:1319–25. doi:10.1038/ng1479
75. Zannas AS, Wiechmann T, Gassen NC, Binder EB. Gene-stress-epigenetic regulation of FKBP5: clinical and translational implications. *Neuropsychopharmacology* (2016) 41:261–74. doi:10.1038/npp.2015.235
76. Zimmermann P, Brückl T, Nocon A, Pfister H, Binder EB, Uhr M, et al. Interaction of FKBP5 gene variants and adverse life events in predicting depression onset: results from a 10-year prospective community study. *Am J Psychiatry* (2011) 168:1107–16. doi:10.1176/appi.ajp.2011.10111577
77. De Bosscher K, Haegeman G. Minireview: latest perspectives on antiinflammatory actions of glucocorticoids. *Mol Endocrinol* (2009) 23:281–91. doi:10.1210/me.2008-0283
78. Desmet SJ, De Bosscher K. Glucocorticoid receptors: finding the middle ground. *J Clin Invest* (2017) 127:1136–45. doi:10.1172/JCI88886
79. Surjit M, Ganti KP, Mukherji A, Ye T, Hua G, Metzger D, et al. Widespread negative response elements mediate direct repression by agonist-liganded glucocorticoid receptor. *Cell* (2011) 145:224–41. doi:10.1016/j.cell.2011.03.027
80. Hudson WH, Youn C, Ortlund EA. The structural basis of direct glucocorticoid-mediated transrepression. *Nat Struct Mol Biol* (2013) 20:53–8. doi:10.1038/nsmb.2456
81. Löwenberg M, Stahn C, Hommes DW, Buttgereit F. Novel insights into mechanisms of glucocorticoid action and the development of new glucocorticoid receptor ligands. *Steroids* (2008) 73:1025–9. doi:10.1016/j.steroids.2007.12.002
82. Boldizsar F, Talaber G, Szabo M, Bartis D, Palinkas L, Nemeth P, et al. Emerging pathways of non-genomic glucocorticoid (GC) signalling in T cells. *Immunobiology* (2010) 215:521–6. doi:10.1016/j.imbio.2009.10.003
83. Croxtall JD, Choudhury Q, Flower RJ. Glucocorticoids act within minutes to inhibit recruitment of signalling factors to activated EGF receptors through a receptor-dependent, transcription-independent mechanism. *Br J Pharmacol* (2000) 130:289–98. doi:10.1038/sj.bjp.0703272
84. Ayroldi E, Cannarile L, Migliorati G, Nocentini G, Delfino DV, Riccardi C. Mechanisms of the anti-inflammatory effects of glucocorticoids: genomic

and nongenomic interference with MAPK signaling pathways. *FASEB J* (2012) 26:4805–20. doi:10.1096/fj.12-216382
85. Scheinman RI, Gualberto A, Jewell CM, Cidlowski JA, Baldwin AS Jr. Characterization of mechanisms involved in transrepression of NF-kappa B by activated glucocorticoid receptors. *Mol Cell Biol* (1995) 15:943–53. doi:10.1128/MCB.15.2.943
86. De Bosscher K, Vanden Berghe W, Haegeman G. The interplay between the glucocorticoid receptor and nuclear factor-kappaB or activator protein-1: molecular mechanisms for gene repression. *Endocr Rev* (2003) 24:488–522. doi:10.1210/er.2002-0006
87. De Bosscher K, Vanden Berghe W, Haegeman G. Cross-talk between nuclear receptors and nuclear factor kappaB. *Oncogene* (2006) 25:6868–86. doi:10.1038/sj.onc.1209935
88. Pascual G, Glass CK. Nuclear receptors versus inflammation: mechanisms of transrepression. *Trends Endocrinol Metab* (2006) 17:321–7. doi:10.1016/j.tem.2006.08.005
89. Jonat C, Rahmsdorf HJ, Park KK, Cato AC, Gebel S, Ponta H, et al. Antitumor promotion and antiinflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. *Cell* (1990) 62:1189–204. doi:10.1016/0092-8674(90)90395-U
90. Helmberg A, Auphan N, Caelles C, Karin M. Glucocorticoid-induced apoptosis of human leukemic cells is caused by the repressive function of the glucocorticoid receptor. *EMBO J* (1995) 14:452–60.
91. Auphan N, DiDonato JA, Rosette C, Helmberg A, Karin M. Immunosuppression by glucocorticoids: inhibition of NF-kappa B activity through induction of I kappa B synthesis. *Science* (1995) 270:286–90. doi:10.1126/science.270.5234.286
92. Vacca A, Felli MP, Farina AR, Martinotti S, Maroder M, Screpanti I, et al. Glucocorticoid receptor-mediated suppression of the interleukin 2 gene expression through impairment of the cooperativity between nuclear factor of activated T cells and AP-1 enhancer elements. *J Exp Med* (1992) 175:637–46. doi:10.1084/jem.175.3.637
93. De Bosscher K, Schmitz ML, Vanden Berghe W, Plaisance S, Fiers W, Haegeman G. Glucocorticoid-mediated repression of nuclear factor-kappaB-dependent transcription involves direct interference with transactivation. *Proc Natl Acad Sci U S A* (1997) 94:13504–9. doi:10.1073/pnas.94.25.13504
94. Liberman AC, Refojo D, Druker J, Toscano M, Rein T, Holsboer F, et al. The activated glucocorticoid receptor inhibits the transcription factor T-bet by direct protein-protein interaction. *FASEB J* (2007) 21:1177–88. doi:10.1096/fj.06-7452com
95. John S, Sabo PJ, Johnson TA, Sung MH, Biddie SC, Lightman SL, et al. Interaction of the glucocorticoid receptor with the chromatin landscape. *Mol Cell* (2008) 29:611–24. doi:10.1016/j.molcel.2008.02.010
96. John S, Sabo PJ, Thurman RE, Sung MH, Biddie SC, Johnson TA, et al. Chromatin accessibility pre-determines glucocorticoid receptor binding patterns. *Nat Genet* (2011) 43:264–8. doi:10.1038/ng.759
97. De Bosscher K, Beck IM, Haegeman G. Classic glucocorticoids versus non-steroidal glucocorticoid receptor modulators: survival of the fittest regulator of the immune system? *Brain Behav Immun* (2010) 24:1035–42. doi:10.1016/j.bbi.2010.06.010
98. De Bosscher K, Haegeman G, Elewaut D. Targeting inflammation using selective glucocorticoid receptor modulators. *Curr Opin Pharmacol* (2010) 10:497–504. doi:10.1016/j.coph.2010.04.007
99. Liberman AC, Castro CN, Antunica Noguerol MA, Barcala Tabarrozzi AE, Druker J, Perone MJ, et al. Immunomodulation by glucocorticoids: from basic research to clinical implications. *Curr Immunol Rev* (2010) 6:371–80.
100. Barnes PJ. Glucocorticosteroids: current and future directions. *Br J Pharmacol* (2011) 163:29–43. doi:10.1111/j.1476-5381.2010.01199.x
101. Vandevyver S, Dejager L, Van Bogaert T, Kleyman A, Liu Y, Tuckermann J, et al. Glucocorticoid receptor dimerization induces MKP1 to protect against TNF-induced inflammation. *J Clin Invest* (2012) 122:2130–40. doi:10.1172/JCI60006
102. Ronchetti S, Migliorati G, Riccardi C. GILZ as a mediator of the anti-inflammatory effects of glucocorticoids. *Front Endocrinol* (2015) 6:170. doi:10.3389/fendo.2015.00170
103. De Bosscher K, Van Craenenbroeck K, Meijer OC, Haegeman G. Selective transrepression versus transactivation mechanisms by glucocorticoid receptor modulators in stress and immune systems. *Eur J Pharmacol* (2008) 583:290–302. doi:10.1016/j.ejphar.2007.11.076
104. Belvisi MG, Wicks SL, Battram CH, Bottoms SE, Redford JE, Woodman P, et al. Therapeutic benefit of a dissociated glucocorticoid and the relevance of in vitro separation of transrepression from transactivation activity. *J Immunol* (2001) 166:1975–82. doi:10.4049/jimmunol.166.3.1975
105. De Bosscher K, Beck IM, Dejager L, Bougarne N, Gaigneaux A, Chateauvieux S, et al. Selective modulation of the glucocorticoid receptor can distinguish between transrepression of NF-kappaB and AP-1. *Cell Mol Life Sci* (2014) 71:143–63. doi:10.1007/s00018-013-1367-4
106. De Bosscher K, Vanden Berghe W, Beck IM, Van Molle W, Hennuyer N, Hapgood J, et al. A fully dissociated compound of plant origin for inflammatory gene repression. *Proc Natl Acad Sci U S A* (2005) 102:15827–32. doi:10.1073/pnas.0505554102
107. Vayssière BM, Dupont S, Choquart A, Petit F, Garcia T, Marchandeau C, et al. Synthetic glucocorticoids that dissociate transactivation and AP-1 transrepression exhibit antiinflammatory activity in vivo. *Mol Endocrinol* (1997) 11:1245–55. doi:10.1210/me.11.9.1245
108. Sugimoto MA, Vago JP, Teixeira MM, Sousa LP. Annexin A1 and the resolution of inflammation: modulation of neutrophil recruitment, apoptosis, and clearance. *J Immunol Res* (2016) 2016:8239258. doi:10.1155/2016/8239258
109. Errasfa M, Russo-Marie F. A purified lipocortin shares the anti-inflammatory effect of glucocorticosteroids in vivo in mice. *Br J Pharmacol* (1989) 97:1051–8. doi:10.1111/j.1476-5381.1989.tb12561.x
110. Buckingham JC, John CD, Solito E, Tierney T, Flower RJ, Christian H, et al. Annexin 1, glucocorticoids, and the neuroendocrine-immune interface. *Ann N Y Acad Sci* (2006) 1088:396–409. doi:10.1196/annals.1366.002
111. Barczyk K, Ehrchen J, Tenbrock K, Ahlmann M, Kneidl J, Viemann D, et al. Glucocorticoids promote survival of anti-inflammatory macrophages via stimulation of adenosine receptor A3. *Blood* (2010) 116:446–55. doi:10.1182/blood-2009-10-247106
112. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. *J Clin Invest* (1998) 101:890–8. doi:10.1172/JCI1112
113. Liu Y, Cousin JM, Hughes J, Van Damme J, Seckl JR, Haslett C, et al. Glucocorticoids promote nonphlogistic phagocytosis of apoptotic leukocytes. *J Immunol* (1999) 162:3639–46.
114. McColl A, Bournazos S, Franz S, Perretti M, Morgan BP, Haslett C, et al. Glucocorticoids induce protein S-dependent phagocytosis of apoptotic neutrophils by human macrophages. *J Immunol* (2009) 183:2167–75. doi:10.4049/jimmunol.0803503
115. Franchimont D. Overview of the actions of glucocorticoids on the immune response: a good model to characterize new pathways of immunosuppression for new treatment strategies. *Ann N Y Acad Sci* (2004) 1024:124–37. doi:10.1196/annals.1321.009
116. Sauer J, Castren M, Hopfner U, Holsboer F, Stalla GK, Arzt E. Inhibition of lipopolysaccharide-induced monocyte interleukin-1 receptor antagonist synthesis by cortisol: involvement of the mineralocorticoid receptor. *J Clin Endocrinol Metab* (1996) 81:73–9. doi:10.1210/jc.81.1.73
117. Ehrchen J, Steinmüller L, Barczyk K, Tenbrock K, Nacken W, Eisenacher M, et al. Glucocorticoids induce differentiation of a specifically activated, anti-inflammatory subtype of human monocytes. *Blood* (2007) 109:1265–74. doi:10.1182/blood-2006-02-001115
118. Vallelian F, Schaer CA, Kaempfer T, Gehrig P, Duerst E, Schoedon G, et al. Glucocorticoid treatment skews human monocyte differentiation into a hemoglobin-clearance phenotype with enhanced heme-iron recycling and antioxidant capacity. *Blood* (2010) 116:5347–56. doi:10.1182/blood-2010-04-277319
119. Ayroldi E, Riccardi C. Glucocorticoid-induced leucine zipper (GILZ): a new important mediator of glucocorticoid action. *FASEB J* (2009) 23:3649–58. doi:10.1096/fj.09-134684
120. Cohen N, Mouly E, Hamdi H, Maillot MC, Pallardy M, Godot V, et al. GILZ expression in human dendritic cells redirects their maturation and prevents antigen-specific T lymphocyte response. *Blood* (2006) 107:2037–44. doi:10.1182/blood-2005-07-2760

121. Elftman MD, Norbury CC, Bonneau RH, Truckenmiller ME. Corticosterone impairs dendritic cell maturation and function. *Immunology* (2007) 122:279–90. doi:10.1111/j.1365-2567.2007.02637.x
122. Hamdi H, Godot V, Maillot MC, Prejean MV, Cohen N, Krzysiek R, et al. Induction of antigen-specific regulatory T lymphocytes by human dendritic cells expressing the glucocorticoid-induced leucine zipper. *Blood* (2007) 110:211–9. doi:10.1182/blood-2006-10-052506
123. Hontelez S, Karthaus N, Looman MW, Ansems M, Adema GJ. DC-SCRIPT regulates glucocorticoid receptor function and expression of its target GILZ in dendritic cells. *J Immunol* (2013) 190:3172–9. doi:10.4049/jimmunol.1201776
124. Luther C, Adamopoulou E, Stoeckle C, Brucklacher-Waldert V, Rosenkranz D, Stoltze L, et al. Prednisolone treatment induces tolerogenic dendritic cells and a regulatory milieu in myasthenia gravis patients. *J Immunol* (2009) 183:841–8. doi:10.4049/jimmunol.0802046
125. Rutella S, Lemoli RM. Regulatory T cells and tolerogenic dendritic cells: from basic biology to clinical applications. *Immunol Lett* (2004) 94:11–26. doi:10.1016/j.imlet.2004.04.015
126. Cao Y, Bender IK, Konstantinidis AK, Shin SC, Jewell CM, Cidlowski JA, et al. Glucocorticoid receptor translational isoforms underlie maturational stage-specific glucocorticoid sensitivities of dendritic cells in mice and humans. *Blood* (2013) 121:1553–62. doi:10.1182/blood-2012-05-432336
127. Cronstein BN, Kimmel SC, Levin RI, Martiniuk F, Weissmann G. A mechanism for the antiinflammatory effects of corticosteroids: the glucocorticoid receptor regulates leukocyte adhesion to endothelial cells and expression of endothelial-leukocyte adhesion molecule 1 and intercellular adhesion molecule 1. *Proc Natl Acad Sci U S A* (1992) 89:9991–5. doi:10.1073/pnas.89.21.9991
128. Pitzalis C, Pipitone N, Bajocchi G, Hall M, Goulding N, Lee A, et al. Corticosteroids inhibit lymphocyte binding to endothelium and intercellular adhesion: an additional mechanism for their anti-inflammatory and immunosuppressive effect. *J Immunol* (1997) 158:5007–16.
129. Pitzalis C, Pipitone N, Perretti M. Regulation of leukocyte-endothelial interactions by glucocorticoids. *Ann N Y Acad Sci* (2002) 966:108–18. doi:10.1111/j.1749-6632.2002.tb04208.x
130. Weber PS, Toelboell T, Chang LC, Tirrell JD, Saama PM, Smith GW, et al. Mechanisms of glucocorticoid-induced down-regulation of neutrophil L-selectin in cattle: evidence for effects at the gene-expression level and primarily on blood neutrophils. *J Leukoc Biol* (2004) 75:815–27. doi:10.1189/jlb.1003505
131. Besedovsky L, Born J, Lange T. Endogenous glucocorticoid receptor signaling drives rhythmic changes in human T-cell subset numbers and the expression of the chemokine receptor CXCR4. *FASEB J* (2014) 28:67–75. doi:10.1096/fj.13-237958
132. Fischer HJ, Schweingruber N, Lühder F, Reichardt HM. The potential role of T cell migration and chemotaxis as targets of glucocorticoids in multiple sclerosis and experimental autoimmune encephalomyelitis. *Mol Cell Endocrinol* (2013) 380:99–107. doi:10.1016/j.mce.2013.04.001
133. Reichardt HM, Kaestner KH, Tuckermann J, Kretz O, Wessely O, Bock R, et al. DNA binding of the glucocorticoid receptor is not essential for survival. *Cell* (1998) 93:531–41. doi:10.1016/S0092-8674(00)81183-6
134. Bouillet P, Metcalf D, Huang DC, Tarlinton DM, Kay TW, Köntgen F, et al. Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity. *Science* (1999) 286:1735–8. doi:10.1126/science.286.5445.1735
135. Knudson CM, Tung KS, Tourtellotte WG, Brown GA, Korsmeyer SJ. Bax-deficient mice with lymphoid hyperplasia and male germ cell death. *Science* (1995) 270:96–9. doi:10.1126/science.270.5233.96
136. Villunger A, Michalak EM, Coultas L, Müllauer F, Böck G, Ausserlechner MJ, et al. p53- and drug-induced apoptotic responses mediated by BH3-only proteins puma and noxa. *Science* (2003) 302:1036–8. doi:10.1126/science.1090072
137. Yin XM, Wang K, Gross A, Zhao Y, Zinkel S, Klocke B, et al. Bid-deficient mice are resistant to Fas-induced hepatocellular apoptosis. *Nature* (1999) 400:886–91. doi:10.1038/23730
138. Purton JF, Monk JA, Liddicoat DR, Kyparissoudis K, Sakkal S, Richardson SJ, et al. Expression of the glucocorticoid receptor from the 1A promoter correlates with T lymphocyte sensitivity to glucocorticoid-induced cell death. *J Immunol* (2004) 173:3816–24. doi:10.4049/jimmunol.173.6.3816
139. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* (1989) 7:145–73. doi:10.1146/annurev.iy.07.040189.001045
140. Farrar JD, Ouyang W, Löhning M, Assenmacher M, Radbruch A, Kanagawa O, et al. An instructive component in T helper cell type 2 (Th2) development mediated by GATA-3. *J Exp Med* (2001) 193:643–50. doi:10.1084/jem.193.5.643
141. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, et al. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell* (2006) 126:1121–33. doi:10.1016/j.cell.2006.07.035
142. Yang XO, Pappu BP, Nurieva R, Akimzhanov A, Kang HS, Chung Y, et al. T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma. *Immunity* (2008) 28:29–39. doi:10.1016/j.immuni.2007.11.016
143. Campbell DJ, Ziegler SF. FOXP3 modifies the phenotypic and functional properties of regulatory T cells. *Nat Rev Immunol* (2007) 7:305–10. doi:10.1038/nri2061
144. Ramírez F, Fowell DJ, Puklavec M, Simmonds S, Mason D. Glucocorticoids promote a TH2 cytokine response by CD4+ T cells in vitro. *J Immunol* (1996) 156:2406–12.
145. Franchimont D, Galon J, Gadina M, Visconti R, Zhou Y, Aringer M, et al. Inhibition of Th1 immune response by glucocorticoids: dexamethasone selectively inhibits IL-12-induced Stat4 phosphorylation in T lymphocytes. *J Immunol* (2000) 164:1768–74. doi:10.4049/jimmunol.164.4.1768
146. Barnes PJ. Th2 cytokines and asthma: an introduction. *Respir Res* (2001) 2:64–5. doi:10.1186/rr39
147. Liberman AC, Druker J, Refojo D, Holsboer F, Arzt E. Glucocorticoids inhibit GATA-3 phosphorylation and activity in T cells. *FASEB J* (2009) 23:1558–71. doi:10.1096/fj.08-121236
148. Maneechotesuwan K, Yao X, Ito K, Jazrawi E, Usmani OS, Adcock IM, et al. Suppression of GATA-3 nuclear import and phosphorylation: a novel mechanism of corticosteroid action in allergic disease. *PLoS Med* (2009) 6:e1000076. doi:10.1371/journal.pmed.1000076
149. Biola A, Andréau K, David M, Sturm M, Haake M, Bertoglio J, et al. The glucocorticoid receptor and STAT6 physically and functionally interact in T-lymphocytes. *FEBS Lett* (2000) 487:229–33. doi:10.1016/S0014-5793(00)02297-3
150. Banuelos J, Cao Y, Shin SC, Lu NZ. Immunopathology alters Th17 cell glucocorticoid sensitivity. *Allergy* (2017) 72:331–41. doi:10.1111/all.13051
151. Ziolkowska M, Koc A, Luszczykiewicz G, Ksiezopolska-Pietrzak K, Klimczak E, Chwalinska-Sadowska H, et al. High levels of IL-17 in rheumatoid arthritis patients: IL-15 triggers in vitro IL-17 production via cyclosporin A-sensitive mechanism. *J Immunol* (2000) 164:2832–8. doi:10.4049/jimmunol.164.5.2832
152. Momcilović M, Miljković Z, Popadić D, Marković M, Savić E, Ramić Z, et al. Methylprednisolone inhibits interleukin-17 and interferon-gamma expression by both naive and primed T cells. *BMC Immunol* (2008) 9:47. doi:10.1186/1471-2172-9-47
153. McKinley L, Alcorn JF, Peterson A, Dupont RB, Kapadia S, Logar A, et al. TH17 cells mediate steroid-resistant airway inflammation and airway hyperresponsiveness in mice. *J Immunol* (2008) 181:4089–97. doi:10.4049/jimmunol.181.6.4089
154. Ramesh R, Kozhaya L, McKevitt K, Djuretic IM, Carlson TJ, Quintero MA, et al. Pro-inflammatory human Th17 cells selectively express P-glycoprotein and are refractory to glucocorticoids. *J Exp Med* (2014) 211:89–104. doi:10.1084/jem.20130301
155. Schewitz-Bowers LP, Lait PJ, Copland DA, Chen P, Wu W, Dhanda AD, et al. Glucocorticoid-resistant Th17 cells are selectively attenuated by cyclosporine A. *Proc Natl Acad Sci U S A* (2015) 112:4080–5. doi:10.1073/pnas.1418316112
156. Banuelos J, Shin S, Cao Y, Bochner BS, Morales-Nebreda L, Budinger GR, et al. BCL-2 protects human and mouse Th17 cells from glucocorticoid-induced apoptosis. *Allergy* (2016) 71:640–50. doi:10.1111/all.12840
157. Zhao J, Lloyd CM, Noble A. Th17 responses in chronic allergic airway inflammation abrogate regulatory T-cell-mediated tolerance and contribute to airway remodeling. *Mucosal Immunol* (2013) 6:335–46. doi:10.1038/mi.2012.76
158. Chen X, Murakami T, Oppenheim JJ, Howard OMZ. Differential response of murine CD4+CD25+ and CD4+CD25- T cells to dexamethasone-induced cell death. *Eur J Immunol* (2004) 34:859–69. doi:10.1002/eji.200324506

159. Chen X, Oppenheim JJ, Winkler-Pickett RT, Ortaldo JR, Howard OM. Glucocorticoid amplifies IL-2-dependent expansion of functional FoxP3(+) CD4(+)CD25(+) T regulatory cells in vivo and enhances their capacity to suppress EAE. *Eur J Immunol* (2006) 36:2139–49. doi:10.1002/eji.200635873
160. Braitch M, Harikrishnan S, Robins RA, Nichols C, Fahey AJ, Showe L, et al. Glucocorticoids increase CD4CD25 cell percentage and Foxp3 expression in patients with multiple sclerosis. *Acta Neurol Scand* (2009) 119:239–45. doi:10.1111/j.1600-0404.2008.01090.x
161. Engler JB, Kursawe N, Solano ME, Patas K, Wehrmann S, Heckmann N, et al. Glucocorticoid receptor in T cells mediates protection from autoimmunity in pregnancy. *Proc Natl Acad Sci U S A* (2017) 114:E181–90. doi:10.1073/pnas.1617115114
162. Wüst S, van den Brandt J, Tischner D, Kleiman A, Tuckermann JP, Gold R, et al. Peripheral T cells are the therapeutic targets of glucocorticoids in experimental autoimmune encephalomyelitis. *J Immunol* (2008) 180:8434–43. doi:10.4049/jimmunol.180.12.8434
163. Olsen PC, Kitoko JZ, Ferreira TP, de-Azevedo CT, Arantes AC, Martins MA. Glucocorticoids decrease Treg cell numbers in lungs of allergic mice. *Eur J Pharmacol* (2015) 747:52–8. doi:10.1016/j.ejphar.2014.11.034
164. Sorrells SF, Caso JR, Munhoz CD, Sapolsky RM. The stressed CNS: when glucocorticoids aggravate inflammation. *Neuron* (2009) 64:33–9. doi:10.1016/j.neuron.2009.09.032
165. Butler LD, Layman NK, Riedl PE, Cain RL, Shellhaas J, Evans GF, et al. Neuroendocrine regulation of in vivo cytokine production and effects: I. In vivo regulatory networks involving the neuroendocrine system, interleukin-1 and tumor necrosis factor-alpha. *J Neuroimmunol* (1989) 24:143–53. doi:10.1016/0165-5728(89)90108-2
166. Goujon E, Parnet P, Cremona S, Dantzer R. Endogenous glucocorticoids down regulate central effects of interleukin-1 beta on body temperature and behaviour in mice. *Brain Res* (1995) 702:173–80. doi:10.1016/0006-8993(95)01041-9
167. Goujon E, Parnet P, Layé S, Combe C, Dantzer R. Adrenalectomy enhances pro-inflammatory cytokines gene expression, in the spleen, pituitary and brain of mice in response to lipopolysaccharide. *Brain Res Mol Brain Res* (1996) 36:53–62. doi:10.1016/0169-328X(95)00242-K
168. Masferrer JL, Seibert K, Zweifel B, Needleman P. Endogenous glucocorticoids regulate an inducible cyclooxygenase enzyme. *Proc Natl Acad Sci U S A* (1992) 89:3917–21. doi:10.1073/pnas.89.9.3917
169. Drew PD, Chavis JA. Inhibition of microglial cell activation by cortisol. *Brain Res Bull* (2000) 52:391–6. doi:10.1016/S0361-9230(00)00275-6
170. Tanaka J, Fujita H, Matsuda S, Toku K, Sakanaka M, Maeda N. Glucocorticoid- and mineralocorticoid receptors in microglial cells: the two receptors mediate differential effects of corticosteroids. *Glia* (1997) 20:23–37. doi:10.1002/(SICI)1098-1136(199705)20:1<23::AID-GLIA3>3.0.CO;2-6
171. Glezer I, Munhoz CD, Kawamoto EM, Marcourakis T, Avellar MC, Scavone C. MK-801 and 7-Ni attenuate the activation of brain NF-kappa B induced by LPS. *Neuropharmacology* (2003) 45:1120–9. doi:10.1016/S0028-3908(03)00279-X
172. Blais V, Turrin NP, Rivest S. Cyclooxygenase 2 (COX-2) inhibition increases the inflammatory response in the brain during systemic immune stimuli. *J Neurochem* (2005) 95:1563–74. doi:10.1111/j.1471-4159.2005.03480.x
173. Blais V, Zhang J, Rivest S. In altering the release of glucocorticoids, ketorolac exacerbates the effects of systemic immune stimuli on expression of proinflammatory genes in the brain. *Endocrinology* (2002) 143:4820–7. doi:10.1210/en.2002-220598
174. Nadeau S, Rivest S. Endotoxemia prevents the cerebral inflammatory wave induced by intraparenchymal lipopolysaccharide injection: role of glucocorticoids and CD14. *J Immunol* (2002) 169:3370–81. doi:10.4049/jimmunol.169.6.3370
175. Nadeau S, Rivest S. Glucocorticoids play a fundamental role in protecting the brain during innate immune response. *J Neurosci* (2003) 23:5536–44. doi:10.1523/JNEUROSCI.23-13-05536.2003
176. Soulet D, Rivest S. Bone-marrow-derived microglia: myth or reality? *Curr Opin Pharmacol* (2008) 8:508–18. doi:10.1016/j.coph.2008.04.002
177. Carrillo-de Sauvage MÁ, Maatouk L, Arnoux I, Pasco M, Sanz Diez A, Delahaye M, et al. Potent and multiple regulatory actions of microglial glucocorticoid receptors during CNS inflammation. *Cell Death Differ* (2013) 20:1546–57. doi:10.1038/cdd.2013.108
178. Frank MG, Miguel ZD, Watkins LR, Maier SF. Prior exposure to glucocorticoids sensitizes the neuroinflammatory and peripheral inflammatory responses to *E. coli* lipopolysaccharide. *Brain Behav Immun* (2010) 24:19–30. doi:10.1016/j.bbi.2009.07.008
179. Johnson JD, O'Connor KA, Deak T, Stark M, Watkins LR, Maier SF. Prior stressor exposure sensitizes LPS-induced cytokine production. *Brain Behav Immun* (2002) 16:461–76. doi:10.1006/brbi.2001.0638
180. Johnson JD, O'Connor KA, Hansen MK, Watkins LR, Maier SF. Effects of prior stress on LPS-induced cytokine and sickness responses. *Am J Physiol Regul Integr Comp Physiol* (2003) 284:R422–32. doi:10.1152/ajpregu.00230.2002
181. Hermoso MA, Matsuguchi T, Smoak K, Cidlowski JA. Glucocorticoids and tumor necrosis factor alpha cooperatively regulate toll-like receptor 2 gene expression. *Mol Cell Biol* (2004) 24:4743–56. doi:10.1128/MCB.24.11.4743-4756.2004
182. Munhoz CD, Lepsch LB, Kawamoto EM, Malta MB, Lima Lde S, Avellar MC, et al. Chronic unpredictable stress exacerbates lipopolysaccharide-induced activation of nuclear factor-kappaB in the frontal cortex and hippocampus via glucocorticoid secretion. *J Neurosci* (2006) 26:3813–20. doi:10.1523/JNEUROSCI.4398-05.2006
183. de Pablos RM, Villarán RF, Argüelles S, Herrera AJ, Venero JL, Ayala A, et al. Stress increases vulnerability to inflammation in the rat prefrontal cortex. *J Neurosci* (2006) 26:5709–19. doi:10.1523/JNEUROSCI.0802-06.2006
184. Espinosa-Oliva AM, de Pablos RM, Villarán RF, Argüelles S, Venero JL, Machado A, et al. Stress is critical for LPS-induced activation of microglia and damage in the rat hippocampus. *Neurobiol Aging* (2011) 32:85–102. doi:10.1016/j.neurobiolaging.2009.01.012
185. Frank MG, Thompson BM, Watkins LR, Maier SF. Glucocorticoids mediate stress-induced priming of microglial pro-inflammatory responses. *Brain Behav Immun* (2012) 26:337–45. doi:10.1016/j.bbi.2011.10.005
186. Frank MG, Watkins LR, Maier SF. Stress- and glucocorticoid-induced priming of neuroinflammatory responses: potential mechanisms of stress-induced vulnerability to drugs of abuse. *Brain Behav Immun* (2011) 25(Suppl 1):S21–8. doi:10.1016/j.bbi.2011.01.005
187. Frank MG, Watkins LR, Maier SF. Stress-induced glucocorticoids as a neuroendocrine alarm signal of danger. *Brain Behav Immun* (2013) 33:1–6. doi:10.1016/j.bbi.2013.02.004
188. Blandino P Jr, Barnum CJ, Solomon LG, Larish Y, Lankow BS, Deak T. Gene expression changes in the hypothalamus provide evidence for regionally-selective changes in IL-1 and microglial markers after acute stress. *Brain Behav Immun* (2009) 23:958–68. doi:10.1016/j.bbi.2009.04.013
189. Frank MG, Baratta MV, Sprunger DB, Watkins LR, Maier SF. Microglia serve as a neuroimmune substrate for stress-induced potentiation of CNS pro-inflammatory cytokine responses. *Brain Behav Immun* (2007) 21:47–59. doi:10.1016/j.bbi.2006.03.005
190. García-Bueno B, Madrigal JL, Pérez-Nievas BG, Leza JC. Stress mediators regulate brain prostaglandin synthesis and peroxisome proliferator-activated receptor-gamma activation after stress in rats. *Endocrinology* (2008) 149:1969–78. doi:10.1210/en.2007-0482
191. Madrigal JL, Hurtado O, Moro MA, Lizasoain I, Lorenzo P, Castrillo A, et al. The increase in TNF-alpha levels is implicated in NF-kappaB activation and inducible nitric oxide synthase expression in brain cortex after immobilization stress. *Neuropsychopharmacology* (2002) 26:155–63. doi:10.1016/S0893-133X(01)00292-5
192. Madrigal JL, Moro MA, Lizasoain I, Lorenzo P, Fernández AP, Rodrigo J, et al. Induction of cyclooxygenase-2 accounts for restraint stress-induced oxidative status in rat brain. *Neuropsychopharmacology* (2003) 28:1579–88. doi:10.1038/sj.npp.1300187
193. O'Connor KA, Johnson JD, Hammack SE, Brooks LM, Spencer RL, Watkins LR, et al. Inescapable shock induces resistance to the effects of dexamethasone. *Psychoneuroendocrinology* (2003) 28:481–500. doi:10.1016/S0306-4530(02)00035-5
194. Ding Y, Gao ZG, Jacobson KA, Suffredini AF. Dexamethasone enhances ATP-induced inflammatory responses in endothelial cells. *J Pharmacol Exp Ther* (2010) 335:693–702. doi:10.1124/jpet.110.171975
195. Lannan EA, Galliher-Beckley AJ, Scoltock AB, Cidlowski JA. Proinflammatory actions of glucocorticoids: glucocorticoids and TNFalpha coregulate gene expression in vitro and in vivo. *Endocrinology* (2012) 153:3701–12. doi:10.1210/en.2012-1020

196. De Bosscher K. Selective glucocorticoid receptor modulators. *J Steroid Biochem Mol Biol* (2010) 120:96–104. doi:10.1016/j.jsbmb.2010.02.027

Conflict of Interest Statement: The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

The reviewer LD and handling Editor declared their shared affiliation.

frontiers in Endocrinology

ORIGINAL RESEARCH
published: 10 July 2018
doi: 10.3389/fendo.2018.00365

Lack of Galectin-3 Disrupts Thymus Homeostasis in Association to Increase of Local and Systemic Glucocorticoid Levels and Steroidogenic Machinery

*Ednéa Oliveira-de-Abreu[1], Danielle Silva-dos-Santos[1], Ailin Lepletier[1†], Tiago D. P. Ramos[1], Rafaella Ferreira-Reis[1], Larissa Vasconcelos-Fontes[1], Mariana T. Ramos[1,2], Rafael C. Torres[2], Vinícius Cotta-de-Almeida[1,3], Vinícius de Frias Carvalho[2,3] and Déa M. S. Villa-Verde[1,3]**

[1] Laboratory on Thymus Research, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, [2] Laboratory of Inflammation, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, [3] National Institute of Science and Technology on Neuroimmunomodulation (INCT-NIM), Rio de Janeiro, Brazil

OPEN ACCESS

Edited by:
Vincent Geenen,
University of Liège, Belgium

Reviewed by:
Gábor B. Makara,
Hungarian Academy of Sciences (MTA), Hungary
Eva Tolosa,
Universitätsklinikum Hamburg-Eppendorf, Germany

***Correspondence:**
Déa M. S. Villa-Verde
dvv@ioc.fiocruz.br;
deavillaverde@gmail.com

†Present Address:
Ailin Lepletier,
Laboratory of Immunology in Cancer and Infection, Department of Immunology, Queensland Institute of Medical Research, Brisbane, Australia

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: 01 February 2018
Accepted: 18 June 2018
Published: 10 July 2018

Citation:
Oliveira-de-Abreu E, Silva-dos-Santos D, Lepletier A, Ramos TDP, Ferreira-Reis R, Vasconcelos-Fontes L, Ramos MT, Torres RC, Cotta-de-Almeida V, Carvalho VF and Villa-Verde DMS (2018) Lack of Galectin-3 Disrupts Thymus Homeostasis in Association to Increase of Local and Systemic Glucocorticoid Levels and Steroidogenic Machinery. Front. Endocrinol. 9:365. doi: 10.3389/fendo.2018.00365

Maintenance of thymus homeostasis is a delicate interplay involving hormones, neurotransmitters and local microenvironmental proteins, as well as saccharides, acting on both thymocytes and stromal cells. Disturbances in these interactions may lead to alterations on thymocyte development. We previously showed that galectin-3, a β-galactoside-binding protein, is constitutively expressed in the thymus, interacting with extracellular matrix glycoproteins and acting as a de-adhesion molecule, thus modulating thymocyte-stromal cell interactions. In this work, we aimed to investigate the participation of galectin-3 in the maintenance of thymus homeostasis, including hormonal-mediated circuits. For that, we used genetically engineered galectin-3-deficient mice. We observed that the thymus of galectin-3-deficient mice was reduced in mass and cellularity compared to wild-type controls; however, the proportions of different thymocyte subpopulations defined by CD4/CD8 expression were not changed. Considering the $CD4^{-}CD8^{-}$ double-negative (DN) subpopulation, an accumulation of the most immature (DN1) stage was observed. Additionally, the proliferative capacity of thymocytes was decreased in all thymocyte subsets, whereas the percentage of apoptosis was increased, especially in the $CD4^{+}CD8^{+}$ double-positive thymocytes. As glucocorticoid hormones are known to be involved in thymus homeostasis, we evaluated serum and intrathymic corticosterone levels by radioimmunoassay, and the expression of steroidogenic machinery using real-time PCR. We detected a significant increase in corticosterone levels in both serum and thymus samples of galectin-3-deficient mice, as compared to age-matched controls. This was paralleled by an increase of gene transcription of the steroidogenic enzymes, steroidogenic acute regulatory protein (*Star*)

and *Cyp11b1* in thymus, 11β-Hydroxysteroid Dehydrogenase (*Hsd11b1)* in the adrenal, and *Cyp11a1* in both glands. In conclusion, our findings show that the absence of galectin-3 subverts mouse thymus homeostasis by a mechanism likely associated to intrathymic and systemic stress-related endocrine circuitries, affecting thymocyte number, proliferation and apoptosis.

Keywords: galectin-3, thymus, thymocytes, proliferation, cell death, glucocorticoid, steroidogenic machinery

INTRODUCTION

Galectins are a family of 15 β-galactoside-binding lectins, containing at least one conserved carbohydrate-recognition domain, which can be found in the nucleus, cytosol, and bound to cell membrane glycoconjugates or to extracellular matrix glycocomponents (1). Depending to their location into the cell, galectins can influence cell proliferation, adhesion, migration, signaling, differentiation, and apoptosis (1, 2). They were also shown to modulate immune functions in health and disease (3, 4). We showed that galectin-3 is constitutively expressed by epithelial and phagocytic cells in both thymic cortex and medulla. Galectin-3 interacts with glycoconjugates on thymocyte surface and extracellular matrix glycoproteins acting as a de-adhesion molecule, thus modulating thymocyte-stromal cell interactions (5). Furthermore, we also noted that galectin-3 accumulates in the thymus of *Trypanosoma cruzi* infected mice, being related to increased thymocyte death and exit to the periphery, and consequent thymus atrophy (6).

Thymus involution is a physiological phenomenon of the organ, related to aging, leading to progressive alterations in the thymus microenvironment, with loss of thymus mass, thymic epithelial cell (TEC) number and function, resulting in a decrease in thymopoiesis. In consequence, a decrease in the immune function is observed in the elderly, with less resistance to infections, autoimmune diseases and cancer (7, 8). On the other hand, acute thymic involution is related to pathological conditions, such as metabolic and infectious diseases (9, 10).

In addition, activation of the hypothalamus–pituitary–adrenal axis induced by stress or some diseases, including diabetes and Chagas disease, was shown to cause severe atrophy of the thymus (11, 12). Glucocorticoids decrease proliferation and increase apoptosis of immature $CD4^+CD8^+$ double-positive (DP) thymocytes, inducing a strong atrophy in the thymus (13, 14). These actions of glucocorticoids on thymus are related to endocrine and paracrine actions of this hormone, since thymus presents the steroidogenic machinery, including StAR, 11β-HSD1, and 11β-HSD2, and is capable to produce glucocorticoids (15, 16).

Considering the multifunctional role of galectin-3 as a regulator of cell adhesion, migration, proliferation, signaling, differentiation and apoptosis, our hypothesis is that galectin-3 is a key player to maintain thymus homeostasis. Here, we undertook this study to evaluate the role of galectin-3 on thymus homeostasis in association with its effects on local and systemic production of glucocorticoids.

MATERIALS AND METHODS

Mice

Male BALB/c wild type (WT) and galectin-3 deficient mice (4–6 week old) were obtained from the Oswaldo Cruz Foundation animal facilities, Rio de Janeiro, Brazil. Galectin-3 deficient mice ($Gal\text{-}3^{-/-}$) were generated by backcrossing with their BALB/c littermates for 9 generations (17). Mice were housed in groups of three in a temperature-, humidity-, and light-controlled (12 h light: 12 h darkness cycle) colony room. Mice were given *ad libitum* access to food and water. All protocols for the use and care of animals were approved by the Ethics Committee for the Use of Experimental Animals of the Oswaldo Cruz Foundation, under licenses number L-024/09 and L-004/2014.

Analysis of T Cell Subpopulations

Individual thymuses were minced, resuspended in phosphate buffered saline solution (PBS) (Sigma Aldrich, St Louis, MO, USA) with 5% Fetal Calf Serum (FCS) (Cultilab, Campinas, SP, Brazil) and counted in Neubauer chamber in the presence of Trypan Blue (Sigma Aldrich) for evaluation of cell viability. Trypan Blue evaluation of cell viability in fresh thymocytes showed that about 95 and 85% of cells were alive in the thymus of WT and $Gal\text{-}3^{-/-}$ mice, respectively. The phenotype of the main thymocyte subpopulations was evaluated by Flow Cytometry with the use of monoclonal antibodies to mouse CD4, CD8, CD44, and CD25 conjugated to different fluorochromes (BD, San Diego, CA, USA). Control isotype immunoglobulins conjugated to correspondent fluorochromes were used for negative staining determination (BD). Briefly, 10^6 thymocytes were incubated for 15 min with 2 μL of normal mouse serum for blockage of unspecific binding, and subsequently with 10 μL of different antibody combinations for 30 min. Cells were then washed in PBS, fixed with 1% formaldehyde and analyzed by Flow Cytometry in a FACSCanto II equipped with the FACSDiva Software (BD). Data were analyzed with the Summit 4.3 Software (Dako Cytomation,Carpinteria, CA, USA).

Evaluation of Thymocyte Proliferation

For evaluation of spontaneous thymocyte proliferation, thymocytes were incubated for 3 h in RPMI medium (Sigma Aldrich) with 10% FCS containing 60 μM bromodeoxyuridine (BrdU) (Sigma Aldrich). Cells were then incubated with anti-CD4, anti-CD8, anti-CD44, anti-CD25 monoclonal antibodies (BD) conjugated to different fluorochromes. After washings, cells were permeabilized using the kit BD Cytofix/Cytoperm™ (BD). Subsequently, BrdU incorporated to cell DNA was exposed by treatment of cells with 100U DNAse I (Roche, Mannheim,

BW, Germany) for 40 min at room temperature. Cells were then washed twice for 5 min at 450 × g, and subsequently incubated with FITC conjugated anti-BrdU (eBioscience, Inc., San Diego, CA, USA). Samples were acquired in a FACSCanto II device (BD) and analyzed with Summit 4.3 Software (Dako Cytomation).

Measurement of Thymocyte Apoptosis

For evaluation of cell death, thymocytes were first stained for surface molecules CD4 and CD8 conjugated to different fluorochromes (BD), washed, suspended in Annexin V buffer and treated for 10 min with 1 μL Annexin V conjugated to fluorescein isothiocyanate (FITC) (BD) according to the manufacturer's recommendations. Cells were immediately analyzed by Flow Cytometry using a FACSCanto II device (BD) equipped with the FACSDiva Software (BD).

Immunofluorescence

The evaluation of thymic epithelial compartment was performed by immunofluorescence. Thymuses (5 animals/group) were removed and frozen in Tissue Tek (Optimal Cutting Temperature Compound, Sakura Finetek, Torrance, CA, USA). Slices of 5 μm-thick thymic sections were obtained in cryostat (Leica CM 1850 - Leica Microsystems Inc.; Buffalo Grove, IL, USA) and fixed in cold acetone for 5 min. Tissue sections were then incubated with 2.5% BSA in PBS for 1 h and subsequently subjected to the indirect immunofluorescence technique for immunolabeling with pan-cytokeratin antibody (Dako), or unrelated control IgG (Molecular Probes, Carlsbad, CA, USA) for 1 h at room temperature. Sections were then washed three times in PBS and incubated for 45 min with the secondary anti-rabbit antibody conjugated to Alexa Fluor 546 (Molecular Probes). Sections were washed again three times in PBS and mounted with Fluoroshield containing 4′,6-diamidino-2-phenylindole - DAPI (Sigma Aldrich) for nuclear staining. Samples were analyzed using a Carl Zeiss Axio Imager Upright Microscope (Zeiss, Oberkochen, BW, Germany).

Histology

Thymus histological analysis was performed by Hematoxylin & Eosin technique. Thymuses (3 animals/group) were fixed in buffered 10% formalin (Millonig buffer) for 48 h. Paraffin-embedded 5-μm sections were mounted on glass slides. The sections were deparaffinized with xylene, and rehydrated by a graded series of ethanol washes. Sections were then left in running water for 1 min, stained with Hematoxylin for 10 min, washed in running water for 1 min, and incubated in Eosin solution for 3 min (Sigma, Aldrich). Photos were taken using the Leica DM 2500 microscope.

Evaluation of Corticosterone Levels

Serum and thymus samples were obtained simultaneously from WT and Gal-$3^{-/-}$ mice.

Animals were euthanized in a CO2 chamber, during the nadir (08:00 h) of the circadian rhythm as described previously (18), and the blood was immediately collected from the abdominal aorta. After blood coagulation, individual sera was collected and stored at −20°C until use. Thymus samples were obtained and kept at a −20°C until use. After thawing, the thymuses were suspended in 150 μl PBS and then triturated in tissue homogenizer. The homogenates were centrifuged at 10,000 × g for 15 min at 4°C. Serum and thymus corticosterone levels were detected by radioimmunoassay (RIA) following manufacturer's guidelines (MP Biomedicals, Solon, OH, USA). Final intrathymic corticosterone levels were represented by the ratio of hormone concentration in supernatants and thymus mass.

Gene Expression of Steroidogenic Enzymes in Adrenals and Thymuses

Thymus and adrenal total RNA from WT and Gal-$3^{-/-}$ animals were obtained using the RNeasy Micro Kit (Qiagen, Valencia, CA, USA). The quantification was performed in the spectrophotometer NanoDrop 1000 (Thermo Ficher Scientific, Waltham, MA, USA). For the synthesis of cDNA, equivalent samples were used in 1 μg of RNA, using SuperScript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA), in the presence of a random primer, according to the manufacturer's recommendation. For the analysis of gene expression by real-time PCR, 100 ng of cDNA samples were diluted in Power SYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, CA, USA) in the Step One Plus system (Applied Biosystems). The PCR method was performed at 95°C for 10 min followed by 40 cycles at 95°C for 15 s, 60°C for 1 min. The specificity of reaction products was verified through the dissociation curve. The data were analyzed by ABI Prism SDS v1.3.1 software. All primers were designed using the Primer Express 3.0 specific program for 7500 FAST Real Time PCR System. cDNA was amplified using specific murine primer sequences described in **Table 1**. After 40 cycles of amplification, expression of cytochrome P450, family 11, subfamily a, polypeptide 1 (*Cyp11a1*), *Cyp11b1*, steroidogenic acute regulatory protein (*Star*), and hydroxysteroid 11-beta dehydrogenase 1 (*Hsd11b1*) was assessed by comparing the expression of each to the normalizer constitutive reference transcript *Rpl-13a* (ribosomal protein L13A), using the Ct method as previously described ($2^{-dCt} \times 1{,}000$) (19), subsequent to the following primer efficiency analysis. Each experiment was run in triplicate with different cDNA preparations from the same mice.

Galectin-3 Inhibition Experiments

In order to investigate the possibility that the lack of galectin-3 is related to thymocyte death observed in our Gal-$3^{-/-}$ mice, we performed *in vitro* experiments using GCS-100, a modified citrus pectin described to act as galectin-3 inhibitor (kindly donated by Dr. S. Patel, La Jolla Pharmaceutical Company, San Diego, CA). Briefly, 10^6 thymocytes of 3-5 WT mice were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 55 μM 2-mercaptoethanol (Gibco, Grand Island, NY), 100 U/mL penicillin, 0.1 mg/mL streptomycin and 0.25 μg/mL amphotericin B (Sigma Aldrich) in the presence of GCS-100, in the concentrations of 50–800 μg/mL, for 24 h at 37°C in a humidified atmosphere containing 5% CO_2. For comparison we included in the experiment thymocytes treated with dexamethasone (0.01 μM). Cells were subsequently incubated with antibodies to CD3, CD4 and CD8 conjugated to

TABLE 1 | Sequence of specific murine primers used for real time qPCR.

Gene (primer)	Sequence
Star	
Forward	5′-TCACTTGGCTGCTCAGTATTGAC-3′
Reverse	5′-GCGATAGGACCTGGTTGATGA-3
Cyp11a1	
Forward	5′-GACCTGGAAGGACCATGCA-3′
Reverse	5′-TGGGTGTACTCATCAGCTTTATTGA-3
Cyp11b1	
Forward	5′-TCAGTCCAGTGTGTTCAACTATACCA-3′
Reverse	5′- GCCGCTCCCCAAAAAGA-3′
Gapdh	
Forward	5′-CCATCACCATCTTCCAGGAG-3′
Reverse	5′-GCATGGACTGTGGTCATGAG-3′
Hsd11d1	
Forward	5′-TGGTGCTCTTCCTGGCCTACT-3′
Reverse	5′-CTGGCCCCAGTGACAATCA-3′
Rpl13	
Forward	5′-CCAAGCAGGTACTTCTGGGCCGGAA-3′
Reverse	5′-CAGTGCGCCAGAAAATGCGGC-3′

different fluorochromes (BD), washed, suspended in Annexin V buffer and treated for 10 min with Annexin-V and 7-AAD for viability evaluation. Cells were immediately analyzed by Flow Cytometry using a FACSCanto II device (BD) equipped with the FACSDiva Software (BD).

Statistical Analysis

Data were evaluated to ensure normal distribution and were statistically analyzed by unpaired *t*-test or ANOVA using the Tukey's multiple comparison test. Data are shown as individual values and median or mean ± standard error (used for real time PCR analysis). Tests were performed using GraphPad Prism 5.0 software (Graphpad Software, San Diego, CA, USA).

RESULTS

Lack of Galectin-3 Induces Thymus Atrophy With Microenvironmental Alterations

We initially observed that galectin-3 deficient mice (Gal-3$^{-/-}$) have a significant thymus atrophy with lower mass and cellularity compared to wild type (WT) BALB/c mice (**Figures 1A–C**). It is important to note that no significant differences were observed considering body mass of both strains of mice. Once the maintenance of adequate thymic architecture is fundamental to thymocyte differentiation, thymus atrophy is frequently accompanied by morphological tissue alterations. So, we evaluated thymus microscopic structure in Gal-3$^{-/-}$ mice using Hematoxylin & Eosin staining. We observed that thymus cortex and medulla are preserved in both WT and Gal-3$^{-/-}$ mice (**Figures 1D**,**E**). However we noticed the presence of concentric structures formed by TEC, similar to Hassall bodies, in the thymic medullary region of Gal-3$^{-/-}$ mice (**Figure 1G**), that are not seen in WT thymuses (**Figure 1F**). We also evaluated the status of the epithelial component of thymic microenvironment by staining thymus sections with anti-pan-cytokeratin antibody. Immunofluorescence data showed a deep disorganization of the thymic epithelial network, with visible TEC-free regions in the thymus of Gal-3$^{-/-}$ mice (**Figures 1I,K**) that were not observed in WT animals (**Figures 1H,J**).

Lack of Galectin-3 Modulates Thymocyte Differentiation

In order to verify if galectin-3 interferes with thymocyte differentiation, we analyzed thymocyte phenotype by flow cytometry using the membrane markers CD4, CD8, CD25 and CD44. We did not find changes in the percentage of thymocyte subpopulations defined by CD4 and CD8 when we compared the two strains of mice (**Figures 2A,B**). However, considering absolute cell numbers, Gal-3$^{-/-}$ mice showed a significant decrease in all thymocyte subpopulations (**Figure 2C**).

The most immature thymocytes are DN for CD4 and CD8, and these cells can be further subdivided in four subsets considering the expression of CD25 and CD44 on their cell membrane. The homeostasis of DN thymocytes is crucial for thymocyte development, as during this stage extensive proliferation, TCR rearrangement and commitment to the αβ or γδ T cell lineages take place. DN1 cells, the most immature DN subpopulation, express CD44 on their cell membrane, but not CD25. Thymocytes sequentially express both CD44 and CD25 (DN2 cells), then lose the expression of CD44 and express only CD25 (DN3 cells), and ultimately are negative for both CD44 and CD25 (DN4 cells; also known as pre-DP) (20). Analysis of DN thymocyte subpopulations in the Gal-3$^{-/-}$ mice showed a significant increase in the percentage of DN1 thymocytes and a decrease in cells in the DN3 stage compared to control WT animals, without alterations in the percentage of DN2 and DN4 cells (**Figures 2D,E**). In absolute numbers, Gal-3$^{-/-}$ mice showed a decrease in all DN subpopulations (**Figure 2F**).

Lack of Galectin 3 Interferes With Thymocyte Proliferation and Death

Proliferation and apoptosis are important events for thymocyte development and maintenance of thymus cellularity. We evaluated if the decrease in thymus mass and cellularity observed in Gal-3$^{-/-}$ mice was related to changes in the rates of both phenomena. Spontaneous thymocyte proliferation, evaluated after 3-h incubation with the thymidine analog BrdU, was decreased both in percentages and absolute numbers in total Gal-3$^{-/-}$ thymocytes when compared to WT (**Figures 3A,B**). Percentages of spontaneous proliferation were decreased in DN, DP and CD4^{+} subpopulations (**Figure 3C**). However, considering absolute numbers DN, DP and CD8^{+}, but not CD4^{+} cells showed decreased proliferation rate (**Figure 3D**). We finally evaluated the levels of spontaneous proliferation in DN subsets of thymocytes and observed a decrease in all DN subpopulations,

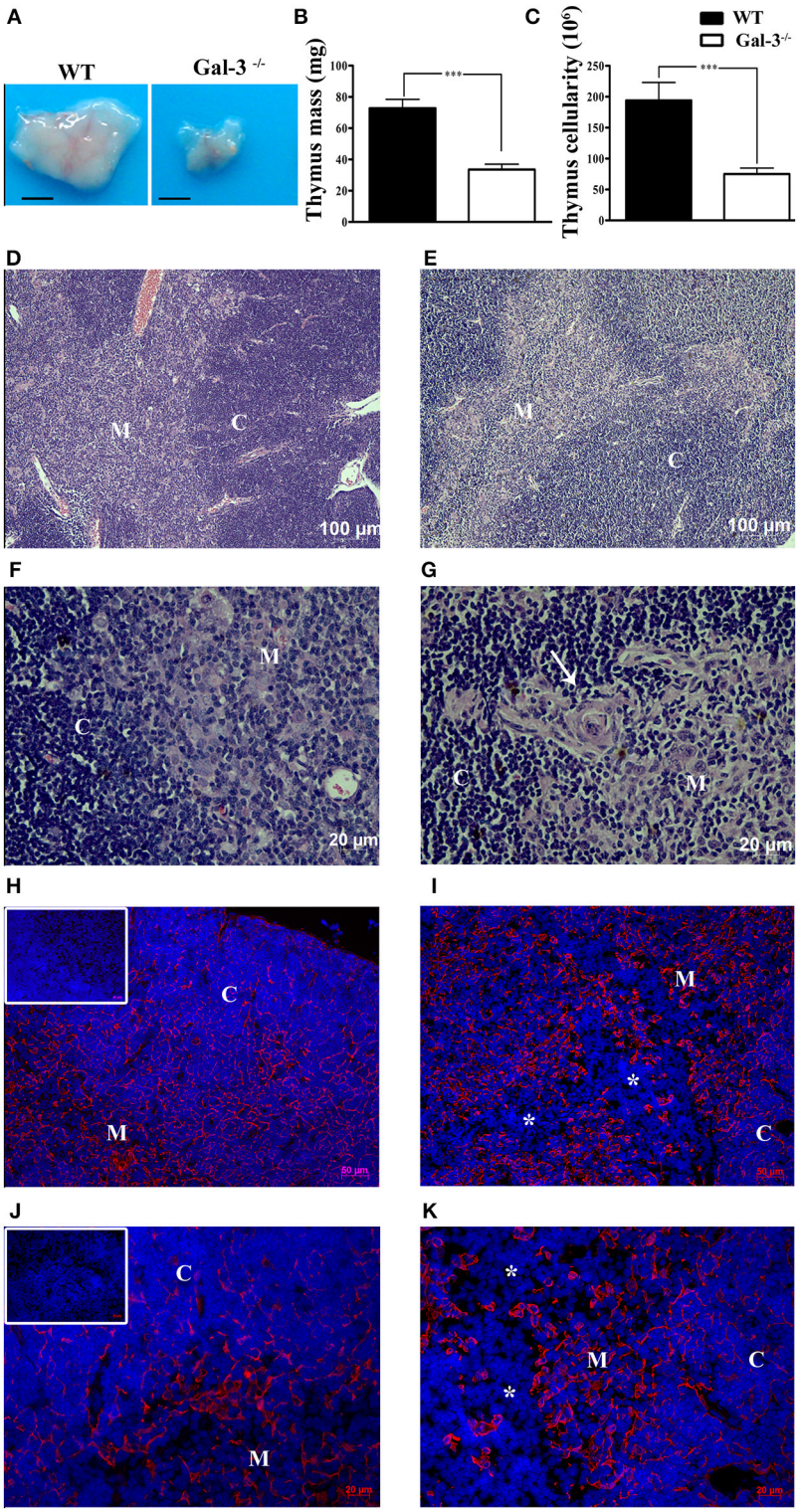

FIGURE 1 | Thymus atrophy and microenvironmental alterations in the absence of galectin-3. **(A)** Shows comparative thymus pictures of WT and Gal-3$^{-/-}$ mice. Thymus mass **(B)** and cellularity **(C)** of Gal-3$^{-/-}$ mice are shown in comparison to WT control mice. Hematoxylin & Eosin stained sections of thymus of WT **(D,F)** and Gal-3$^{-/-}$ **(E,G)** mice. Presence of concentric Hassall body-like structures is shown in Gal-3$^{-/-}$ thymus (white arrow in **G**) but not in WT mice **(F)**. Immunofluorescence staining with anti-pan-cytokeratin is shown in the thymus of WT **(H,J)** and Gal-3$^{-/-}$ **(I,K)** mice. Inserts in **(H,J)** show negative controls. Asterisks in **(I**,**K)** denote DAPI stained TEC-free regions. Blue staining in panels: DAPI, used to show cell nuclei. Images are representative of 5 animals/group. Bar in **(A)**: 0.25 cm. C, Cortex; M, Medulla.

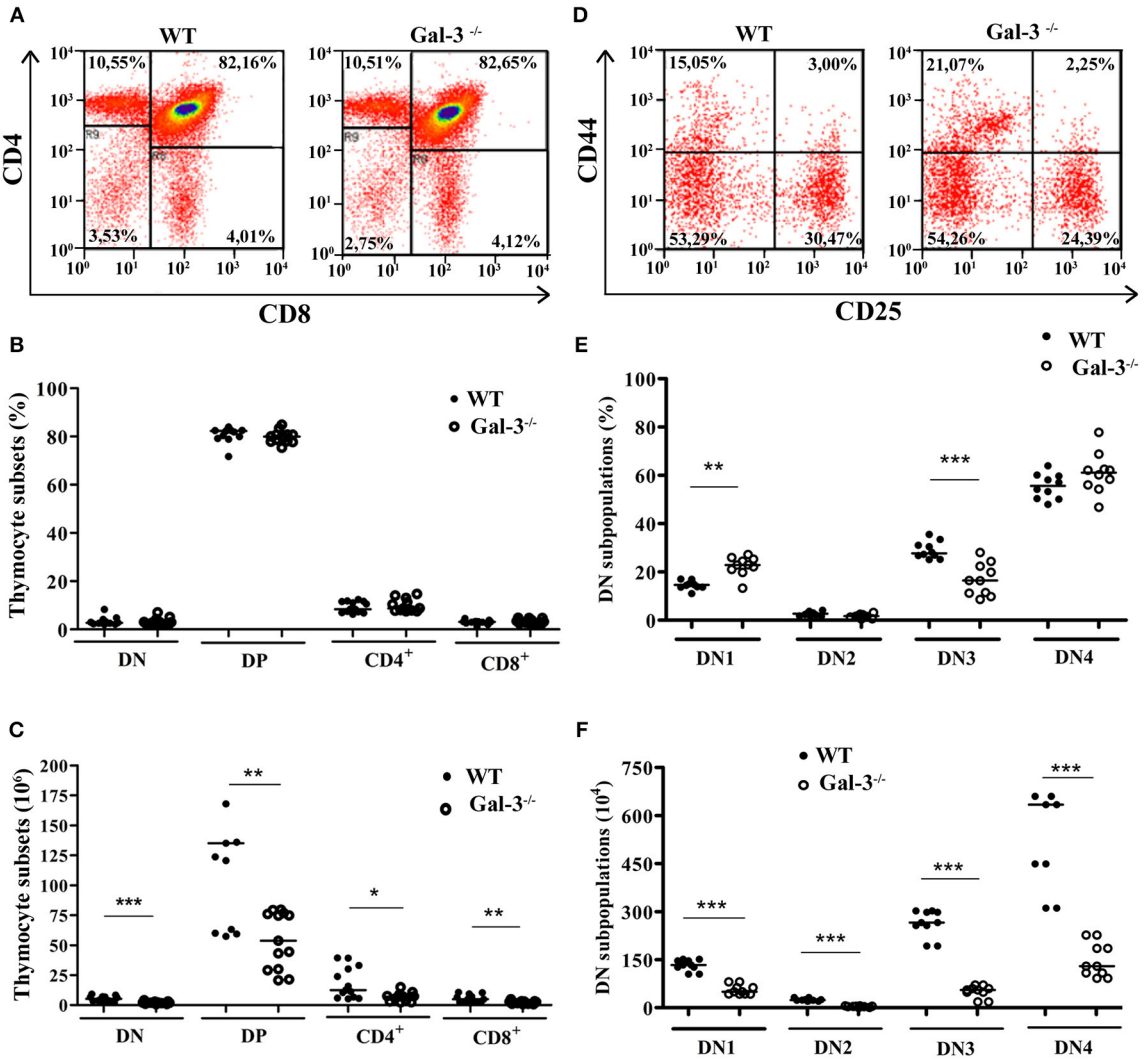

FIGURE 2 | Modulation of thymocyte differentiation in the absence of galectin-3. **(A)** Shows representative dot plots obtained after CD4/CD8 staining of WT and Gal-3$^{-/-}$ thymocytes. **(B,C)** Respectively show percentage and absolute numbers of thymocyte subpopulations defined by CD4/CD8 staining of WT and Gal-3$^{-/-}$ mice. Data are representative of 14 animals/group. **(D)** Shows representative dot plots for CD44/CD25 staining of WT and Gal-3$^{-/-}$ thymocytes. **(E,F)** Respectively show percentage and absolute numbers of DN thymocyte subpopulations defined by CD44/CD25 staining of WT and Gal-3$^{-/-}$ mice. Data are representative of 10 animals/group. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

from DN1 to DN4, both in percentage and absolute numbers (**Figures 3E,F**).

The results of cell death analysis, evaluated after labeling cells with Annexin V, showed statistically significant increase in the percentage of Annexin V$^+$ DP thymocytes of Gal-3$^{-/-}$ mice, compared to WT animals (**Figure 3G**). No differences were observed in absolute numbers (**Figure 3H**).

Lack of Galectin-3 Increases Serum and Intrathymic Corticosterone Levels

Considering the participation of glucocorticoids on thymus involution, we initially evaluated the levels of corticosterone in the serum and thymus of both strains of mice. We detected high corticosterone levels in both serum and thymus of Gal-3$^{-/-}$ mice compared to WT animals (**Figure 4**). In adrenal glands, Gal-3$^{-/-}$ mice presented an increase in the expression of steroidogenic enzyme genes *Cyp11a1* (**Figure 5B**) and *Hsd11b1* (**Figure 5D**), but did not alter the gene expression of *Star* (**Figure 5A**) and *Cyp11b1* (**Figure 5C**), while in the thymus of Gal-3$^{-/-}$ mice we noticed higher expression of *Star* (**Figure 5E**), *Cyp11a1* (**Figure 5F**), and *Cyp11b1* (**Figure 5G**), and no difference in *Hsd11b1* (**Figure 5H**) gene expression.

Inhibition of Galectin-3 in WT Mice Increases Thymocyte Apoptosis

To determine in which extent the increased thymocyte apoptosis observed in Gal-3$^{-/-}$ mice was due to the primary lack of galectin-3 or to the high corticosterone

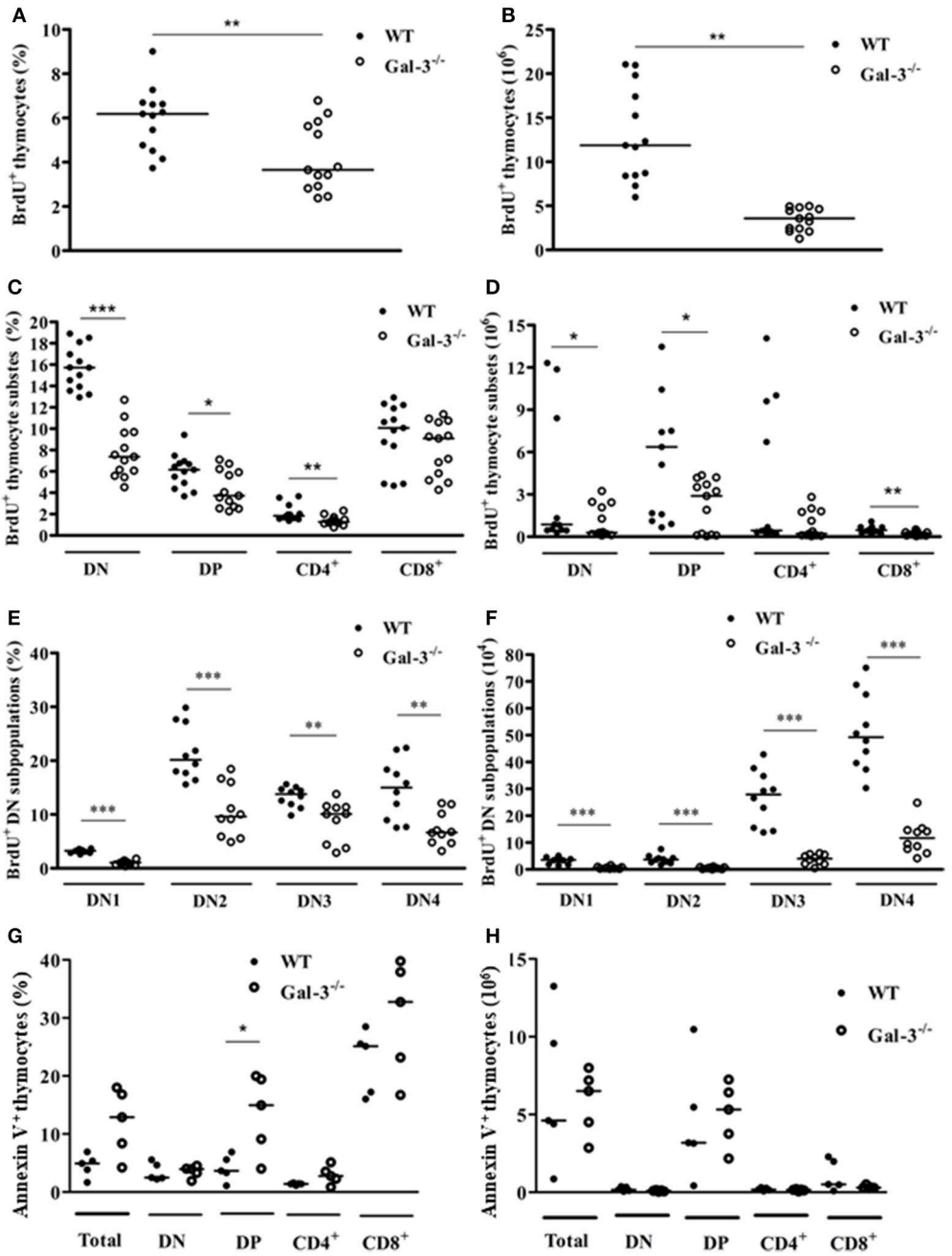

FIGURE 3 | Changes in thymocyte proliferation and death in the absence of galectin-3. BrdU incorporation by total thymocytes of WT and Gal-3$^{-/-}$ mice is shown in percentage **(A)** and absolute cell numbers **(B)**. **(C**,**D)** Respectively show BrdU incorporation by CD4/CD8-defined thymocyte subpopulations in percentage and absolute numbers. **(E**,**F)** Respectively show BrdU incorporation by DN thymocyte subpopulations in percentage and absolute numbers. Data are representative of 10 animals/group. **(G**,**H)** Respectively show percentage and absolute numbers of Annexin V^{+} cells in total and CD4/CD8-defined thymocyte subpopulations. Data are representative of 5 animals/group. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

levels observed in these animals, we treated thymocytes obtained from WT mice with different concentrations of GCS-100, a modified citrus pectin described to function as a galectin-3 inhibitor (21, 22), or dexamethasone *in vitro* for 24 h. Our results showed that thymocytes treated with different concentrations of GCS-100 for 24 h were more susceptible to apoptosis than untreated cells, as shown by the staining with 7-AAD/Annexin V (**Figure 6**). Moreover, DP thymocytes were the most susceptible cells comparing different thymocyte subpopulations. As expected, dexamethasone-treated thymocytes were induced to apoptosis, mainly DP cells (**Figure 6E**).

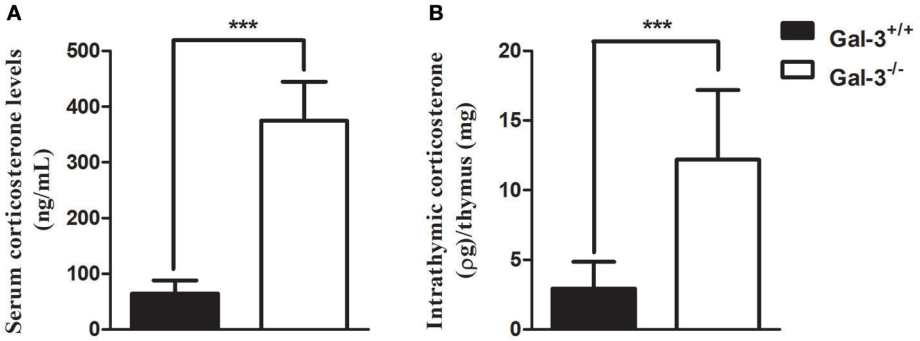

FIGURE 4 | Increase in serum and intrathymic corticosterone levels in the absence of galectin-3. Radioimmune assay analysis of serum **(A)** and intrathymic **(B)** corticosterone levels of WT and Gal-3$^{-/-}$ mice. Final intrathymic corticosterone levels were represented by the ratio of hormone concentration in supernatants and thymus mass. Data are representative of 10 animals/group. ***$p < 0.001$.

DISCUSSION

Thymocyte differentiation from bone marrow-derived precursors is dependent on their interactions with the thymic microenvironment, composed of stromal cells, namely TEC, fibroblasts, macrophages, dendritic cells; extracellular matrix (ECM) molecules, represented by fibronectin, laminin, type IV collagen; and soluble proteins, such as cytokines, chemokines, galectins, and with the neuro and endocrine systems (12, 23). In the present study we showed that the lack of galectin-3 consistently affected thymus homeostasis in association to local and systemic glucocorticoid production. Initially, we noticed that thymus mass and cellularity were significantly decreased in Gal-3$^{-/-}$ mice compared to WT, presenting also alterations in the epithelial component of the thymic microenvironment, with regions without TEC in the thymus. These alterations in the structure of thymus epithelial network observed in Gal-3$^{-/-}$ mice could, at least partly, explain the reduction of cellularity in this organ, once we previously demonstrated that galectin-3 is expressed by epithelial cells of both thymus cortex and medulla, playing a de-adhesive role by modulating thymocyte interactions with stromal cells and ECM components (5). Furthermore, the present data suggest that galectin-3 is not only important for the interactions of thymocytes with the thymic stroma, but also to the maintenance of thymic architecture and thymocyte homeostasis.

Next, we showed that the absolute numbers of different thymocyte subsets, defined by CD4 and CD8 molecules, were affected in Gal-3$^{-/-}$ mice. The lack of galectin-3 seems to affect all thymocyte subpopulations, impacting the organ as a whole. Interestingly, considering DN thymocytes, the most immature subset in which important events of thymocyte differentiation occur, we observed an accumulation of DN1 and a decrease in DN3 cells, whereas in absolute numbers all DN subpopulations were decreased. In fact, we detected that thymocytes of Gal-3$^{-/-}$ mice proliferate significantly less than those of WT, and this reduction was importantly noted in DN thymocytes. These alterations may affect all thymocyte development, leading to decreased thymus mass and cellularity, as in the DN stage extensive proliferation, TCR rearrangement and commitment to the αβ or γδ T cell lineages were described to happen (20). Our data, pointing the DN subset as strongly affected in the absence of galectin-3, might be related to critical interactions of the lectin with its ligands within specific niches in the subcapsular or cortical zones of the thymus. Further mechanistic studies are warranted to elucidate such a possibility.

It is also important to mention that in Gal-3$^{-/-}$ mice, thymocyte death was increased in DP subset, the most numerous of the thymocyte subpopulations. Different data in the literature showed that galectin-3, a multifunctional molecule included in the class of matricellular proteins (1, 24, 25), acts both extracellularly, where it participates in cell adhesion and migration, and intracellularly, being able to regulate proliferation, apoptosis and cell signaling (3). In fact, galectin-3 was shown to protect cells from apoptosis, as it has the NWGR motif highly conserved in the BH1 domain of the Bcl-2 gene family, a well characterized suppressor of apoptosis, and was shown to interact with Bcl-2 (26, 27). Moreover, the expression of galectin-3 was shown to be upregulated in proliferating fibroblasts, suggesting a possible role for this lectin in the regulation of cell growth (28). Considering the important thymus atrophy noted in Gal-3$^{-/-}$ mice, and that immature thymocytes are extremely sensitive to glucocorticoids we evaluated the levels of serum and thymus GC in these mice. Our results showed that Gal-3$^{-/-}$ mice present extremely higher levels of serum and thymus corticosterone compared to WT. Previous works described the endocrine and paracrine actions of GC on thymocyte physiology, and that thymus presents the steroidogenic machinery, including StAR, 11β-HSD1 and 11β-HSD2 and is able to produce GC itself (15, 16), and express GC receptors (29, 30). Indeed, GC were shown to decrease proliferation and increase apoptosis of immature thymocytes (13, 14). Furthermore, blockage of GC receptors was shown to partially revert thymus atrophy observed in *Trypanosoma cruzi* infected mice (31). We showed here that the production of GC seems to be modulated by the lack of galectin-3, with

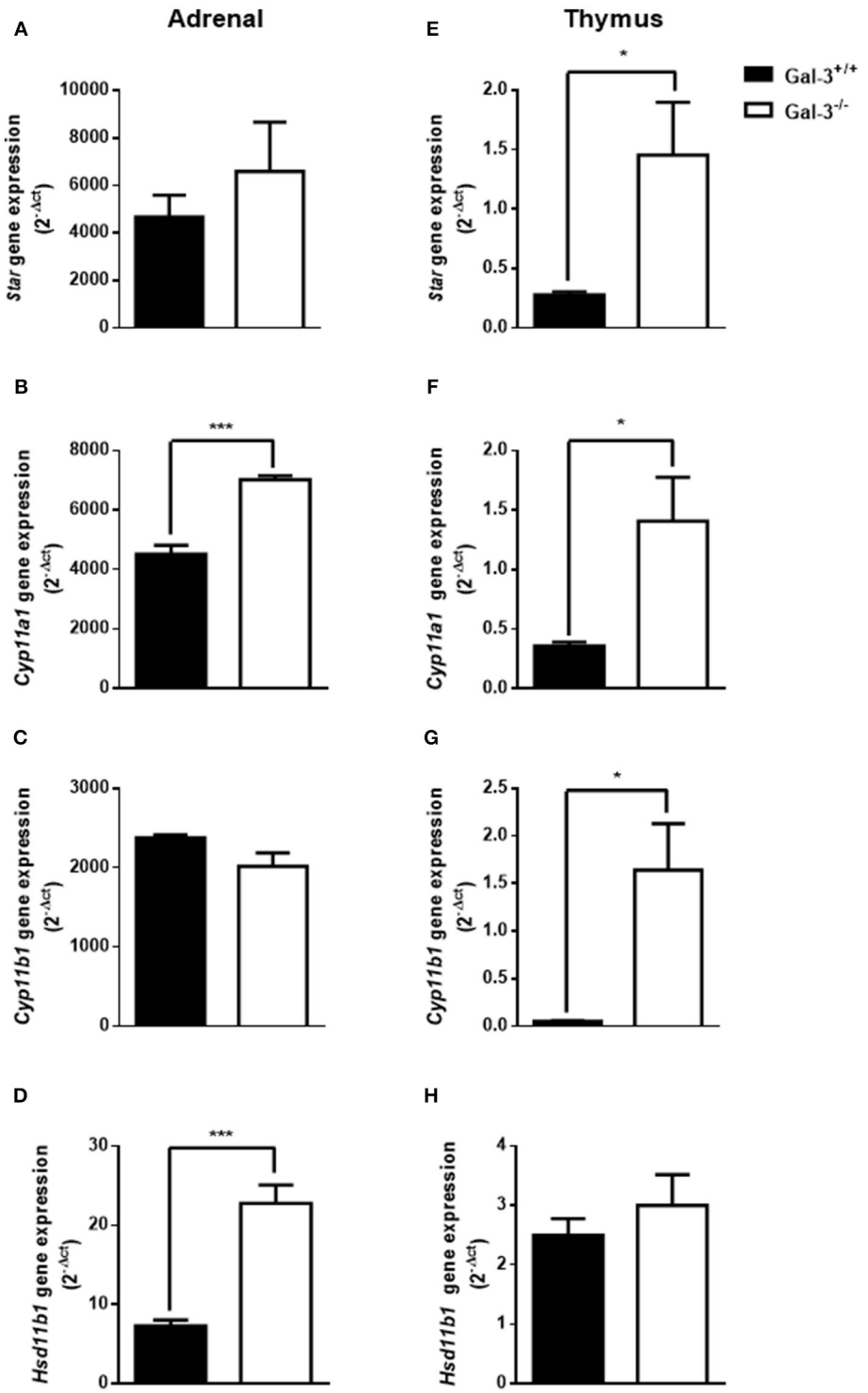

FIGURE 5 | Changes of steroidogenic machinery in the absence of galectin-3. Gene expression of steroidogenic enzymes *Star* **(A,E)**, *Cyp11a1* **(B,F)**, *Cyp11b1***(C,G)**, and *Hsd11b1* **(D,H)** in adrenals and thymuses of WT and Gal-3$^{-/-}$ mice was measured by qPCR. The values were normalized to the constitutive reference transcript *Rpl-13a*. Values are represented as (2-dct) of gene expression are shown as mean ± standard error. Each experiment was run in triplicate with different cDNA preparations from the same mice. Data are representative of 4 animals/group. *$p < 0.05$; ***$p < 0.001$.

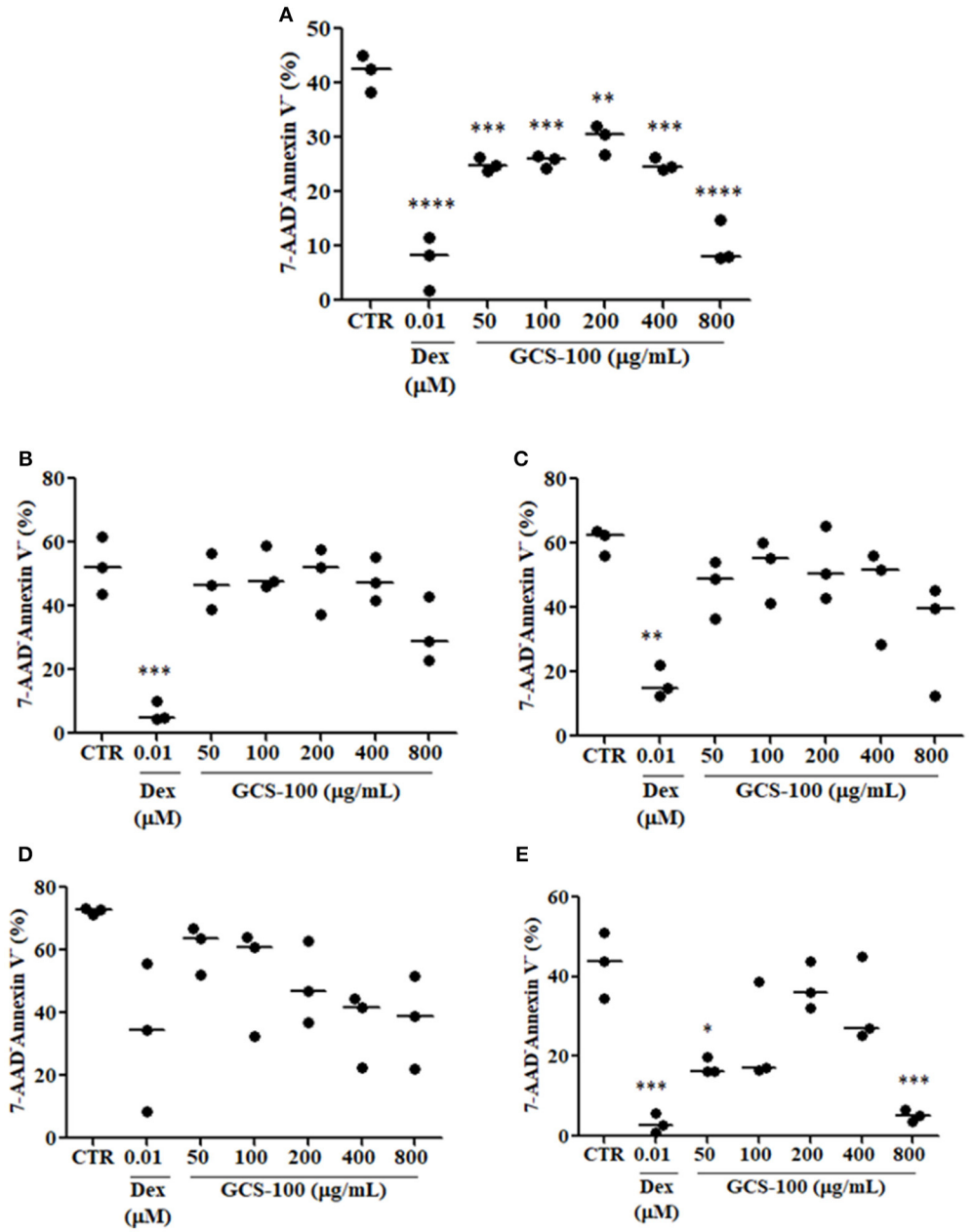

FIGURE 6 | Inhibition of galectin-3 increases thymocyte apoptosis. **(A)** Shows % of living cells (7-AAD$^-$/Annexin V$^-$) in total thymocytes treated for 24 h with different concentrations of GCS-100 (50–800 μg/mL) or dexamethasone (0.01 μM). **(B–E)** Respectively show % of living cells (7-AAD$^-$/Annexin V$^-$) in CD4SP, CD8SP, DN/CD3$^-$ and DP thymocyte subpopulations submitted to the same treatments. Data show one representative of two independent experiments with 3 animals/group, with similar results. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

concomitant increase in steroidogenic machinery both in the adrenals and thymus. We believe that high GC content in Gal-3$^{-/-}$ mice may be involved in thymus atrophy, namely increased immature thymocyte death and decreased proliferation. We must also keep in mind that the lack of galectin-3, an anti-apoptotic molecule, may also be involved in the increased thymocyte death observed in Gal-3$^{-/-}$ mice. To elucidate how much of the changes on thymocyte cellularity are due to hormonal changes and how much are due to the lack of galectin-3, we treated thymocytes from WT mice *in vitro* with a galectin-3 inhibitor (GCS-100) or with dexamethasone, and evaluated cell susceptibility to apoptosis. GCS-100 was previously shown to induce apoptosis in different cell lines and to regulate susceptibility to cell death (21, 22, 32). We showed here that thymocytes treated with different doses of GCS-100 were more susceptible to cell death *in vitro*. Moreover, double-positive

thymocytes were the most affected cells, as observed also after dexamethasone treatment. From our new data, we suggest that both the increased GC contents and the lack of galectin-3 are likely to contribute to thymus atrophy in Gal-3$^{-/-}$ mice. Taking together our *in vivo* and *in vitro* results, it is important to point out the divergence regarding cell death susceptibility of double-positive subset in the absence of galectin-3. Further studies approaching *in vivo* treatment of thymocytes with GCS-100 inhibitor are needed to clarify this point. Another important issue to be considered is the production of other galectins by thymic epithelial cells described to induce apoptosis in thymocytes, namely galectins −1, −8 and −9 (33–36). It is possible that the lack of the anti-apoptotic galectin-3, together with the presence of the pro-apoptotic galectins −1, −8 and −9 would unbalance thymocyte homeostasis, favoring thymocytes to be more sensitive to pro-apoptotic effects of other galectins secreted by the thymus microenvironment and even to apoptotic factors such as GC.

We should also have in mind that the lack of galectin-3 during all lifespan of Gal-3$^{-/-}$ mice may have pleiotropic effects, influencing different organs that in sequence may affect the thymus. In fact, the lack of galectin-3 was shown to cause changes in the bone marrow, an important contributor to thymus cellularity with the generation of T cell precursors. This tissue was shown to be drastically modified in Gal-3$^{-/-}$ mice with reduced cell density (37). Alterations on B cell precursors were defined, but nothing was described for thymocyte precursors (38). A detailed study on T cell precursors of Gal-3$^{-/-}$ mice is missing.

Interesting changes in the thymus microenvironment were also observed in Gal-3$^{-/-}$ mice in relation to WT, such as the appearance of concentric structures similar to Hassall bodies, which are not commonly seen in young mouse thymuses. Hassall bodies are formed by TEC expressing high molecular weight cytokeratins, and represent advanced stages of TEC maturation (39). These alterations may be related to the high corticosterone contents observed in our mice, as GC hormones are able to induce senescence and changes in cytokeratin and ECM expression in TECs (40–42). The existence of a galectin-3/GC circuitry has not been well established up to now and demands further studies. It is not clear if the levels of GC hormones interfere with galectin-3 secretion and vice-versa. However, previous studies showed that mice submitted to stress or macrophages treated with GC have decreased galectin-3 expression (43–45).

Another intriguing alteration that called our attention in the thymus of Gal-3$^{-/-}$ mice was the presence of TEC-free regions in the thymic cortex, that were not observed in WT mice. Similar data were described previously in the thymus of aged mice and could be restored by oral zinc supplementation, as this chemical element is fundamental to thymus homeostasis, influencing the production of the thymic hormone thymulin, as well as TEC and thymocyte development (46). Cortical TEC-free regions have also been described in the thymus of different lupus strains of mice (NZB, MRL/MP-lpr/lpr, BXSB/MpJ Yaa, and C3H/HeJ-gld/gld), which undergo premature involution, but not in normal strains, including BALB/c, C57BL/6, and DBA (47). Changes in the thymic microenvironment, such as the occurrence of cortical TEC-free areas, may be harmful for T cell maturation, including positive selection that is dependent on cortical TEC/thymocyte interactions, reflecting in the accumulation of immature thymocytes and decreased thymus cellularity. Although no data relate these "TEC-free" regions to dysregulated selective events in the thymus, it is possible that the disturbed TEC-thymocyte interactions observed in Gal-3$^{-/-}$ mice contributes to the decrease in thymus cellularity.

CONCLUSION

Our data suggest that the lack of galectin-3 unbalances the steroidogenic machinery homeostasis in both thymus and adrenal, leading to an increase in local and systemic GC production, which in turn contributes to thymus atrophy by increasing thymocyte apoptosis and reducing thymocyte proliferation and TEC function. Besides, the direct effects of galectin-3 absence, such as defective intrathymic thymocyte migration, impaired proliferation and increased susceptibility to apoptosis, must be considered in the scenario of thymus atrophy observed in Gal-3$^{-/-}$mice.

AUTHOR CONTRIBUTIONS

EO performed all experiments and analyses, participated in study design and manuscript writing, DS participated in flow cytometry and immunofluorescence studies and analyses, AL participated in radioimmunoassay and performed qPCR analyses, MR participated in histological analyses, TR participated in immunofluorescence and proliferation studies, RF-R performed GCS-100 experiments, LV-F performed immunofluorescence analysis, RT participated in radioimmunoassay, VC-A participated in data interpretation and wrote the manuscript, VC performed radioimmunoassay studies, participated in data interpretation and wrote the manuscript, DV-V designed the study, participated in data interpretation and wrote the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

ACKNOWLEDGMENTS

This work was supported by grants from CNPq (484235/2007-9 and 311242/2013-8), FAPERJ (E-26 111.373/2013), Oswaldo Cruz Institute, (Brazil) and Mercosur Fund for Structural Convergence (FOCEM) #03/11. The authors thank Dr. Sima Patel (La Jolla Pharmaceutical Company, San Diego, CA) for the kind donation of GCS-100.

REFERENCES

1. Elola MT, Wolfenstein-Todel C, Troncoso MF, Vasta GR, Rabinovich GA. Galectins: matricellular glycan-binding proteins linking cell adhesion, migration, and survival. *Cell Mol Life Sci.* (2007) 64:1679–700. doi: 10.1007/s00018-007-7044-8
2. Liu FT, Patterson RJ, Wang JL. Intracellular functions of galectins. *Biochim Biophys Acta* (2002) 1572:263–73. doi: 10.1016/S0304-4165(02)00313-6
3. Dumic J, Dabelic S, Flögel M. Galectin-3: an open-ended story. *Biochim Biophys Acta* (2006) 1760:616–35. doi: 10.1016/j.bbagen.2005.12.020
4. Rabinovich GA, Toscano MA. Turning "sweet" on immunity: Galectin-glycan interactions in immune tolerance and inflammation. *Nat Rev Immunol.* (2009) 9:338–52. doi: 10.1038/nri2536
5. Villa-Verde DM, Silva-Monteiro E, Jasiulionis MG, Farias-De-Oliveira DA, Brentani RR, Savino W, et al. Galectin-3 modulates carbohydrate-dependent thymocyte interactions with the thymic microenvironment. *Eur J Immunol.* (2002) 32:1434–44. doi: 10.1002/1521-4141(200205)32:5<1434::AID-IMMU1434>3.0.CO;2-M
6. Silva-Monteiro E, Reis Lorenzato L, Kenji Nihei O, Junqueira M, Rabinovich GA, Hsu DK, et al. Altered expression of galectin-3 induces cortical thymocyte depletion and premature exit of immature thymocytes during Trypanosoma cruzi infection. *Am J Pathol.* (2007) 170:546–56. doi: 10.2353/ajpath.2007.060389
7. Taub DD, Longo DL. Insights into thymic aging and regeneration. *Immunol Rev.* (2005) 205:72–93. doi: 10.1111/j.0105-2896.2005.00275.x
8. Lynch HE, Goldberg GL, Chidgey A, Van den Brink MR, Boyd R, Sempowski GD. Thymic involution and immune reconstitution. *Trends Immunol.* (2009) 30:366–73. doi: 10.1016/j.it.2009.04.003
9. Gruver AL, Sempowski GD. Cytokines, leptin, and stress-induced thymic atrophy. *J Leukoc Biol.* (2008) 84:915–23. doi: 10.1189/jlb.0108025
10. De Meis J, Aurélio Farias-De-Oliveira D, Nunes Panzenhagen PH, Maran N, Villa-Verde DM, Morrot A, et al. Thymus atrophy and double-positive escape are common features in infectious diseases. *J Parasitol Res.* (2012) 2012:574020. doi: 10.1155/2012/574020
11. Savino W, Dardenne M. Immune-neuroendocrine interactions. *Immunol Today* (1995) 16:318–22. doi: 10.1016/0167-5699(95)80144-8
12. Savino W, Mendes-Da-Cruz DA, Lepletier A, Dardenne M. Hormonal control of T-cell development in health and disease. *Nat Rev Endocrinol.* (2016) 12:77–89. doi: 10.1038/nrendo.2015.168
13. Cohen JJ. Glucocorticoid-induced apoptosis in the thymus. *Semin Immunol.* (1992) 4:363–9.
14. Berki T, Pálinkás L, Boldizsár F NP. Glucocorticoid (GC) sensitivity and GC receptor expression differ in thymocyte subpopulations. *Int Immunol.* (2002) 14:463–9.
15. Vacchio MS, Lee JY, Ashwell JD. Thymus-derived glucocorticoids set the thresholds for thymocyte selection by inhibiting TCR-mediated thymocyte activation. *J Immunol.* (1999) 163:1327–33.
16. Lepletier A, de Carvalho VF, Rodrigues e Silva PM, Villar S, Pérez AR, Savino W, et al. Trypanosoma cruzi disrupts thymic homeostasis by altering intrathymic and systemic stress-related endocrine circuitries. *PLoS Negl Trop Dis.* (2013) 7:e2470. doi: 10.1371/journal.pntd.0002470.
17. Hsu DK, Yang RY, Pan Z, Yu L, Salomon DR, Fung-Leung WP, et al. Targeted disruption of the galectin-3 gene results in attenuated peritoneal inflammatory responses. *Am J Pathol.* (2000) 156:1073–83. doi: 10.1016/S0002-9440(10)64975-9
18. Torres RC, Magalhães NS, Rodrigues e Silva PM, Martins MA, Carvalho VF. Activation of PPAR-γ reduces HPA axis activity in diabetic rats by up-regulating PI3K expression. *Exp Mol Pathol.* (2016) 101:290–301. doi: 10.1016/j.yexmp.2016.10.002
19. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. *Methods* (2001) 25:402–8. doi: 10.1006/meth.2001.1262
20. Ciofani M, Zúñiga-Pflücker JC. The thymus as an inductive site for T lymphopoiesis. *Annu Rev Cell Dev Biol.* (2007) 23:463–93. doi: 10.1146/annurev.cellbio.23.090506.123547
21. Chauhan D, Li G, Podar K, Hideshima T, Neri P, He D, et al. A novel carbohydrate-based therapeutic GCS-100 overcomes bortezomib resistance and enhances dexamethasone-induced apoptosis in multiple myeloma cells. *Cancer Res.* (2005) 65:8350–8. doi: 10.1158/0008-5472.CAN-05-0163
22. Streetly MJ, Maharaj L, Joel S, Schey SA, Gribben JG, Cotter FE. GCS-100, a novel galectin-3 antagonist, modulates MCL-1, NOXA, and cell cycle to induce myeloma cell death. *Blood* (2010) 115:3939–48. doi: 10.1182/blood-2009-10-251660
23. Savino W, Mendes-da-Cruz DA, Smaniotto S, Silva-Monteiro E, Villa-Verde DM. Molecular mechanisms governing thymocyte migration: combined role of chemokines and extracellular matrix. *J Leukoc Biol.* (2004) 75:951–61. doi: 10.1189/jlb.1003455
24. Sage EH, Bornstein P. Extracellular proteins that modulate cell-matrix interactions. SPARC, tenascin, and thrombospondin. *J Biol Chem.* (1991) 266:14831–4.
25. Levy Y, Arbel-Goren R, Hadari YR, Eshhar S, Ronen D, Elhanany E, et al. Galectin-8 functions as a matricellular modulator of cell adhesion. *J Biol Chem.* (2001) 276:31285–95. doi: 10.1074/jbc.M100340200
26. Yang RY, Hsu DK, Liu FT. Expression of galectin-3 modulates T-cell growth and apoptosis. *Proc Natl Acad Sci U S A.* (1996) 93:6737–42. doi: 10.1073/pnas.93.13.6737
27. Akahani S, Nangia-Makker P, Inohara Hi, Kim H-R Ci, Raz A. Galectin-3 : a novel antiapoptotic molecule with a functional BH1 (NWGR) A-J. *Cancer Res.* (1997) 57:5272–6.
28. Moutsatsos IK, Wadet M, Schindler M, Wang JL. Endogenous lectins from cultured cells : nuclear localization of carbohydrate-binding protein 35 in proliferating 3T3 fibroblasts. *Proc Natl Acad Sci U S A.* (1987) 84:6452–6456.
29. Tsawdaroglou NG, Govindan MV, Schmid W SC. Dexamethasone-binding proteins in cytosol and nucleus of rat thymocytes purification of three receptor proteins. *Eur J Biochem.* (1981) 114:305–13. doi: 10.1111/j.1432-1033.1981.tb05150.x
30. Ranelletti FO, Maggiano N, Aiello FB, Carbone A, Larocca LM, Musiani P, et al. Glucocorticoid receptors and corticosensitivity of human thymocytes at discrete stages of intrathymic differentiation. *J Immunol.* (1987) 138:440–5.
31. Farias-de-Oliveira DA, Villa-Verde DM, Panzenhagen PH, Silva dos Santos D, Berbert LR, Savino W, et al. Caspase-8 and caspase-9 mediate thymocyte apoptosis in trypanosoma cruzi acutely infected mice. *J Leukoc Biol.* (2013) 93:227–34. doi: 10.1189/jlb.1211589
32. Clark MC, Pang M, Hsu DK, Liu F, de Vos S, Gascoyne RD, et al. Galectin-3 binds to CD45 on diffuse large B-cell lymphoma cells to regulate susceptibility to cell death. *Blood* (2012) 120:4635–45. doi: 10.1182/blood-2012-06-438234
33. Baum LG, Pang M, Perillo NL, Wu T, Delegeane A, Uittenbogaart CH, et al. Human thymic epithelial cells express an endogenous lectin, galectin-1, which binds to core 2 O-glycans on thymocytes and T lymphoblastoid cells. *J Exp Med.* (1995) 181:877–87.
34. Tribulatti MV, Mucci J, Cattaneo V, Agücero F, Gilmartin T, Head SR, et al. Galectin-8 Induces apoptosis in the CD4highCD8high thymocyte subpopulation. *Glycobiology* (2007) 17:1404–12. doi: 10.1093/glycob/cwm104
35. Wada J, Ota K, Kumar A, Wallner EI, Kanwar YS. Developmental regulation, expression, and apoptotic potential of galectin-9, a beta-galactoside binding lectin. *J Clin Invest.* (1997) 99:2452–61. doi: 10.1172/JCI119429
36. Bi S, Earl LA, Jacobs L, Baum LG. Structural features of galectin-9 and galectin-1 that determine distinct T cell death pathways. *J Biol Chem.* (2008) 283:12248–58. doi: 10.1074/jbc.M800523200
37. Brand C, Oliveira FL, Ricon L, Fermino ML, Boldrini LC, Hsu DK, et al. The bone marrow compartment is modified in the absence of galectin-3. *Cell Tissue Res.* (2011) 346:427–37. doi: 10.1007/s00441-011-1276-5
38. De Oliveira FL, Dos Santos SN, Ricon L, Da Costa TP, Pereira JX, Brand C, et al. Lack of galectin-3 modifies differentially Notch ligands in bone marrow and spleen stromal cells interfering with B cell differentiation. *Sci Rep.* (2018) 8:1–14. doi: 10.1038/s41598-018-21409-7
39. Nicolas JF, Reano A, Kaiserlian D, Thivolet J. Epithelial cell heterogeneity in mammalian thymus: monoclonal antibody to high molecular weight keratins exclusively binds to Hassall's corpuscles. *Histochem J.* (1989) 21:357–64.
40. Talaber G, Kvell K, Varecza Z, Boldizsar F, Parnell SM, Jenkinson EJ, et al. Wnt-4 Protects Thymic Epithelial Cells Against Dexamethasone-Induced Senescence. *Rejuvenation Res.* (2011) 14:241–8. doi: 10.1089/rej.2010.1110
41. Cirne-Lima EO SW. Repeated *in vivo* hydrocortisone treatment promotes a dual modulation of cytokeratin expression by mouse thymic epithelial cells. *Neuroimmunomodulation* (1998) 5:328–31. doi: 10.1159/000026353

42. Lannes-Vieira J, Dardenne M, Savino W. Extracellular matrix components of the mouse thymus microenvironment: ontogenetic studies and modulation by glucocorticoid hormones. *J Histochem Cytochem.* (1991) 39:1539–46. doi: 10.1177/39.11.1918928
43. Dumić J1, Barisić K, Flögel M, Lauc G. Galectin-3 decreases in mice exposed to immobilization stress. *Stress* (2000) 3:241–6.
44. Maldonado CA, Sundblad V, Salatino M, Elia O, García LN, Leimgruber C, et al. Cell-type specific regulation of galectin-3 expression by glucocorticoids in lung Clara cells and macrophages. *Histol Histopathol.* (2011) 26:747–59. doi: 10.14670/HH-26.747
45. Dabelic S, Supraha S, Dumic J. Galectin-3 in macrophage-like cells exposed to immunomodulatory drugs. *Biochim Biophys Acta* (2006) 1760:701–9. doi: 10.1016/j.bbagen.2005.11.020
46. Dardenne M, Boukaiba N, Gagnerault MC, Homo-Delarche F, Chappuis P, Lemonnier D et al. Restoration of the thymus in aging mice by *in vivo* zinc supplementation. *Clin Immunol Immunopathol.* (1993) 66:127–35. doi: 10.1006/clin.1993.1016
47. Takeoka Y, Yoshida SH, Van de Water J, Boyd R, Suehiro S, Ansari AA, et al. Thymic microenvironmental abnormalities in MRL/MP-lpr/lpr, BXSB/MpJ Yaa and C3H HeJ-gld/gld mice. *J Autoimmun.* (1995) 8:145–61.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

PERSPECTIVE
published: 03 April 2018
doi: 10.3389/fendo.2018.00135

Can a Proper T-Cell Development Occur in an Altered Thymic Epithelium? Lessons From EphB-Deficient Thymi

*Juan José Muñoz[1], Javier García-Ceca[2], Sara Montero-Herradón[2], Beatriz Sánchez del Collado[2], David Alfaro[2] and Agustín Zapata[1,2]**

[1] Center for Cytometry and Fluorescence Microscopy, Complutense University of Madrid, Madrid, Spain, [2] Department of Cell Biology, Faculty of Biology, Complutense University of Madrid, Madrid, Spain

OPEN ACCESS

Edited by:
Wilson Savino, Fundação Oswaldo Cruz (Fiocruz), Brazil

Reviewed by:
Nils Lambrecht, VA Long Beach Healthcare System, University of California Irvine, United States
Elena Gonzalez-Rey, Institute of Parasitology and Biomedicine "López-Neyra" (CSIC), Spain

***Correspondence:**
Agustín Zapata
zapata@ucm.es

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: *19 December 2017*
Accepted: *15 March 2018*
Published: *03 April 2018*

Citation:
Muñoz JJ, García-Ceca J, Montero-Herradón S, Sánchez del Collado B, Alfaro D and Zapata A (2018) Can a Proper T-Cell Development Occur in an Altered Thymic Epithelium? Lessons From EphB-Deficient Thymi. Front. Endocrinol. 9:135. doi: 10.3389/fendo.2018.00135

For a long time, the effects of distinct Eph tyrosine kinase receptors and their ligands, ephrins on the structure, immunophenotype, and development of thymus and their main cell components, thymocytes (T) and thymic epithelial cells (TECs), have been studied. In recent years, the thymic phenotype of mutant mice deficient in several Ephs and ephrins B has been determined. Remarkably, thymic stroma in these animals exhibits important defects that appear early in ontogeny but little alterations in the proportions of distinct lymphoid cell populations. In the present manuscript, we summarize and extend these results discussing possible mechanisms governing phenotypical and functional thymocyte maturation in an absence of the critical T–TEC interactions, concluding that some signaling mediated by key molecules, such as MHCII, CD80, β5t, Aire, etc. could be sufficient to enable a proper maturation of thymocytes, independently of morphological alterations affecting thymic epithelium.

Keywords: thymus, thymocytes, thymic epithelial cells, Eph, ephrins

INTRODUCTION

The organogenesis of complex tissues requires the coordinated differentiation of cells at the correct time and place. A central role for Eph kinase receptors and their ligands, ephrins in these processes, has been claimed (1, 2) and, particularly, in the thymus (3). Both Eph and ephrins provide positional information for cells, regulating cell-to-cell contacts, cell migration, cell survival and differentiation. Eph receptors include EphA (10 members) that preferentially, but not exclusively, bind ephrins-A (6 members) and EphB (6 members) that interact with ephrins-B (3 members). In this system, Eph/ephrin binding results in a bidirectional signaling, forward in the case of Eph and reverse in that of the ephrins (4). Both molecules that partially govern the establishment of the neural network in the central nervous system (5) also modulate the thymocyte migration throughout the thymic compartments (6) and temporal and topological thymocyte (T)–thymic epithelial cell (TECs) interactions (3, 7). The relevance of such cell-to-cell interactions has been classically recognized, but some recent data question its importance (8). In the present analysis, we limit the relevance of T–TEC crosstalk to explain the absence of a clear thymocyte phenotype in thymi exhibiting a severely altered thymic epithelial network.

THE THYMIC PHENOTYPE OF EphB-DEFICIENT MICE

Phenotypes of thymocytes and TECs are remarkably different in EphB2 and EphB3 knockout thymi. Three major features characterize these thymi: hypocellularity, profound alterations in the morphology and histology of TECs, and few changes occurring in the proportions of distinct thymocyte subpopulations. In addition, these phenotypes appear early in the thymus ontogeny and gradually increase when T–TEC interactions become more intense in WT thymi.

Absence of EphB2 and/or EphB3 Courses With Low Number of Both Thymocytes and TECs

The lack of Eph or ephrins courses with thymic hypocellularity that affect both thymocytes (9, 10) and TECs (11, 12), and the blockade of Eph/ephrin signaling reduces thymic cell numbers (13, 14).

Low numbers of recent emigrants seeding the mutant thymi and their slow maturation (10, 15) are a major cause of the thymic hypocellularity, in addition to their increased apoptosis and reduced proportions of cycling thymocytes (9). Reduced proportions of cycling thymocytes could be associated with decreased Delta-like 4 (Dll4) and IL7 receptor α chain transcript (12) as is also observed in thymocytes exhibiting specific deletion of ephrin-B1 and ephrin-B2 (16), both molecules involved in the maturation of developing double-negative (DN) thymocytes (17, 18).

Reduced lymphoid immigrants affect, by turn, the proportions of immature MTS20$^+$ TECs (10) contributing to their delayed maturation and decreased TEC numbers. Also, altered proportions of cycling TECs and apoptotic TECs account for TEC hypocellularity of mutant thymi (12). Fetal and postnatal thymi of EphB2- and/or EphB3-deficient mice show higher proportions of apoptotic TECs than WT ones, which correlates with a reduced thymic K8$^+$ epithelial network (19). At E12.5, cell proliferation is delayed in Eph-deficient TECs as a consequence of the delayed seeding of lymphoid progenitor cells into mutant thymic primordium (10, 12). Later, decreased proportions of cycling cells, which could be partially related to decreased transcripts of FGF7 and its receptor FGFR2IIIb (12) involved in thymic epithelium proliferation (20, 21), have been related with the delayed maturation of mutant thymic epithelium.

Delayed Maturation of TEC Subsets Also Occurs in EphB-Deficient Thymi

Important morphological and immunohistochemical changes occur in the epithelial cell subpopulations of EphB-deficient thymi, including the presence of K5$^+$K8$^+$MTS10$^+$ immature medullary TECs (mTECs), high numbers of K5$^-$K8$^-$MTS20$^+$ cells and K5$^+$K8$^+$ cortical TECs (cTECs) and increased number and size of K5$^-$K8$^-$ epithelial-free areas (11, 22). By flow cytometry, we confirmed delayed maturation of immature MTS20$^+$ TECs, cTECs defined by the expression of Ly51, CD205, MHCII, CD40 and β5t, and mTECs identified by UEA-1, Cld3/4, SSEA-1, MHCII, CD40, CD80, and Aire medullary markers (12, Montero-Herradón et al 2017, submitted manuscript)[1]. This defective epithelial maturation culminates in the aberrant phenotypes of mutant adult thymi, in which the 3D epithelial network is disrupted by the inability of thymocytes and TECs to adequately intermingle.

On the other hand, although it has been reported that the absence of one or several Eph has no phenotype because of the known promiscuity of this molecular system, in which practically any Eph and ephrin can interact thus favoring a certain overlapping (23, 24), we recently demonstrated a specificity in the effects of EphB2 and EphB3 on TECs. Remarkably, although both EphB2 and EphB3 are necessary for a proper development of both cortical and medullary epithelia, the lack of EphB2 results in a more severe medulla phenotype than that of EphB3$^{-/-}$ mTEC (Montero-Herradón et al 2017, submitted manuscript)[1], whereas the thymic cortex of EphB3$^{-/-}$ mice is particularly affected (12).

Morphological Changes in the Mutant TECs

The absence of EphB specifically affects the TEC morphology. In the medulla, mTECs undergo a shortening of cell processes appearing as globular cells in both EphB2$^{-/-}$ and EphB3$^{-/-}$ thymi, but in the cortex EphB2$^{-/-}$ cTECs show reduced cell processes resulting in a rounded cell shape, whereas EphB3$^{-/-}$ cTECs exhibit long, perpendicular cell processes. Independently of the changes undergone, mutant cells appear considerably separated from both thymocytes and other TECs (11). In order to confirm whether the lack of either EphB2 or EphB3 affected TEC shape, reaggregate thymus organ cultures (RTOCs) formed from WT fetal thymus lobes treated (or not) with either blocking anti-EphB2 or anti-EphB3 antibodies were examined. In treated RTOCs, TEC morphology was similar to that of the respective mutant thymi: rounded in those treated with anti-EphB2 and exhibiting long, perpendicular cell processes in those receiving anti-EphB3 (**Figures 1A,B**). In these conditions, epithelial cell processes were significantly shorter in both EphB2$^{-/-}$ or anti-EphB2-treated RTOCs and longer in EphB3$^{-/-}$ and anti-EphB3-treated RTOCs, than in WT RTOCs (**Figures 1C,D**).

It is largely known that the Eph/ephrin signaling modulates cytoskeleton and cell adhesion (25). More specifically, in RTOCs, the blockade of Eph signaling by soluble ephrin-B1Fc provokes TEC rounding with the disappearance of cell processes and disorganization of the cytoskeleton (13). In agreement, EphB2 and EphB3 regulate the morphology of neuronal dendrite spines (26), the lack of ephrin-B2 elongates muscle cells and induces lamellipodium formation (27), and bone marrow-derived mesenchymal stromal cells treated with EphB2-Fc or EphB4-Fc fusion proteins undergo roundness and reduced size (28). It is evident, therefore, that morphological changes in TECs of EphB-deficient thymi hinder the establishment of proper cell-to-cell contacts between thymocytes and TECs, critical for the adequate maturation of both thymic cell components, as previously indicated for the increased apoptotic TECs found in mutant thymi.

[1]Montero-Herradon S, Garcia-Ceca J, Zapata AG. Delayed maturation of mTEC in EphB-deficient thymi is recovered by RANK signaling stimulation. Manuscript submitted (2017).

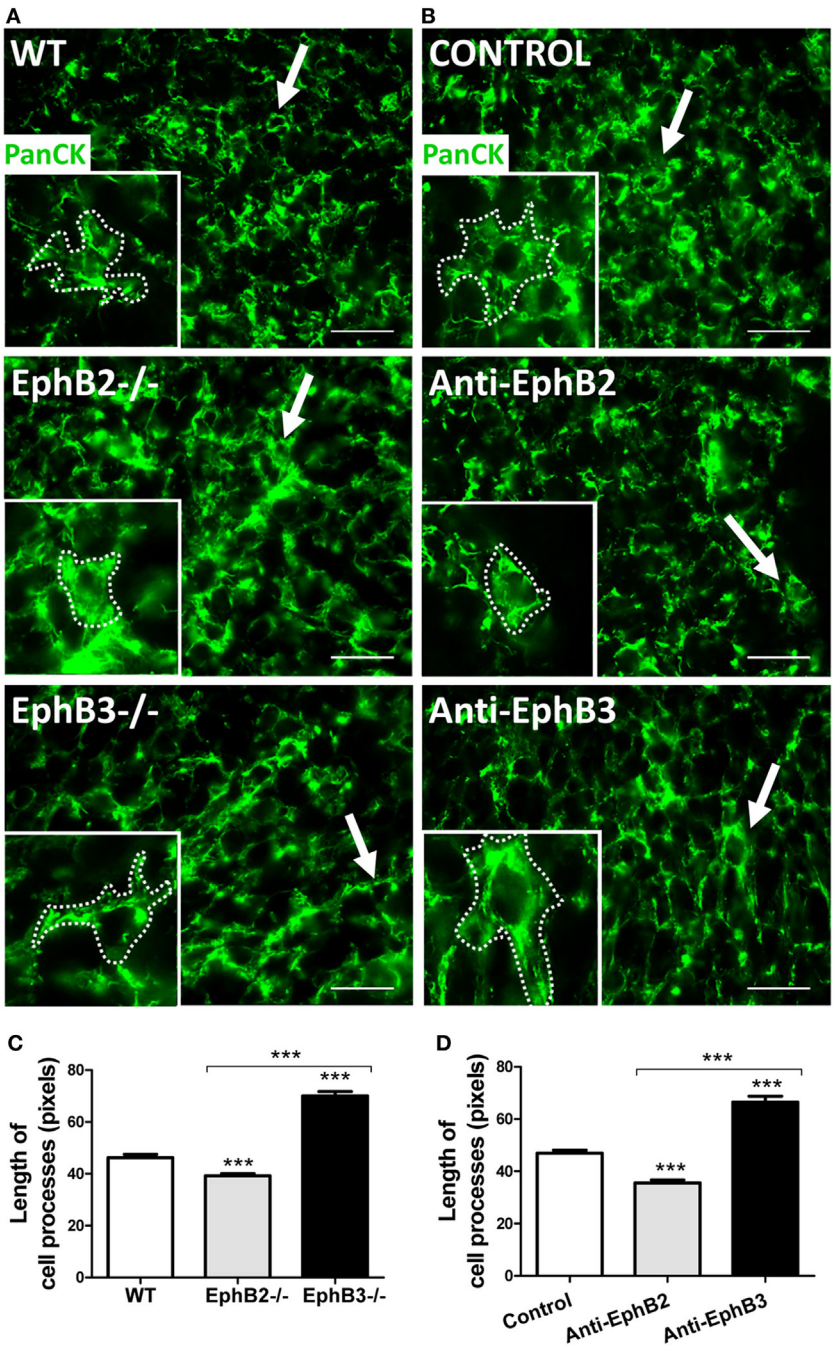

FIGURE 1 | Thymic epithelial cell (TEC) morphology in reaggregate thymus organ cultures (RTOCs) established with either WT cells or EphB-deficient cells, or RTOCs treated with either blocking anti-EphB2 or anti-EphB3 antibodies. **(A,B)** Standard immunofluorescence study of the TEC network stained with an anti-PanCytokeratin antibody (PanCK, Green) and details of the TEC morphology in the different established RTOCs. Notice the shortened epithelial cell processes (arrows and insert dotted line) in RTOCs established with EphB2$^{-/-}$ cells **(A)** or treated with a blocking anti-EphB2 antibody **(B)** and the elongated cell processes (arrows and insert dotted line) in RTOCs established with EphB3$^{-/-}$ cells **(A)** or treated with anti-EphB3 antibody, as compared with their respective WT controls **(A)** or isotype control antibodies [**(B)**, control]. The inserts illustrate the morphology of these cells. Scale: 50 μm. **(C,D)** Morphometric analysis of the length of cell processes in RTOCs established with either EphB2- or EphB3-deficient cells or RTOCs treated with blocking anti-EphB2 or anti-EphB3 antibodies. Note the reduced length of cell processes in RTOCs established with EphB2$^{-/-}$ cells **(C)** or treated with anti-EphB2 antibody **(D)**, while those established with EphB3$^{-/-}$ cells **(C)** or treated with anti-EphB3 antibody **(D)** show longer cell processes as compared with their control RTOCs. The length of cell processes was measured in pixels in those cells whose cell body appeared sectioned. Five RTOCs of each experimental group were studied measuring about 25 cells and a total of 100–150 cell processes by reaggregate. The significance of the Student's *t*-test probability is indicated as $^{***}p \leq 0.005$.

Histological Organization of Thymic Cortex and Medulla

In the thymic medulla of both fetal EphB2- and EphB3-deficient thymi, there are profound modifications that after birth, they remain in EphB2$^{-/-}$ medulla and improve partially in EphB3$^{-/-}$ thymi. Mature thymic medulla is organized from individual islets that expand and fuse after birth (29). By contrast, in mutant thymi, particularly in EphB2$^{-/-}$ ones, a unique adult thymic medulla is impaired and only small isolated foci remain. Adult EphB3-deficient thymi have a more organized central medulla but small, scattered medullary foci also appear (11). Furthermore, the quantification of these medullary foci in RTOCs established again with either WT, EphB2$^{-/-}$, EphB3$^{-/-}$, blocking anti-EphB2- or anti-EphB3-treated fetal thymus lobes confirmed the existence of more and significantly smaller foci in mutant and treated lobes, with differences between those deficient in EphB2 and EphB3 (Montero-Herradón et al 2017, submitted manuscript)[1].

THE CONDITION OF T CELLS IN EphB-DEFICIENT MICE

Although lower numbers of lymphoid progenitor cells seed both mutant adult and embryonic thymi than in WT mice (10, 15), their subsequent progression does not result in notable changes in the percentages of distinct thymocyte subsets. Some delayed maturation of the DN cell subpopulations occurs with increased proportions of total DN cells and unchanged values of both double-positive (DP) cells and single-positive thymocytes. Within the DN cell populations, DN1 cells increased, whereas DN3 cell compartment underwent a significant reduction (9, 10). Decreased proportions of DN3 thymocytes could be associated with changes in TCR selection or molecules involved in their maturation, such as Dll4 and IL7 whose transcripts diminish in EphB-deficient thymi (12). In addition, Luo and colleagues (16) reported a reduced expression of the IL7 receptor α chain in ephrin-B1/ephrin-B2-deleted thymocytes.

On the other hand, analysis of the TCR repertoire of mutant CD4$^+$ cells by using a battery of antibodies specific to different TCR rearrangements only found increased proportions of Vβ3$^+$CD4$^+$ cells in both thymus and lymph nodes (30), and the peripheral lymphoid organs (peripheral blood, spleen, lymph nodes) did not exhibit an altered architecture, a disturbed topological distribution of lymphoid and macrophage areas (30) nor significant changes in the proportions of CD4/CD8 T lymphocyte subpopulations (9). Presumably, the mesenchyme-derived stroma of both spleen and lymph nodes is less affected than TECs by the lack of EphB. On the other hand, no changes occur in the proportions of TH1 (TCRαβ$^+$CD4$^+$IFNγ$^+$), TH2 (TCRαβ$^+$CD4$^+$IL4$^+$), and TH17 (TCRαβ$^+$CD4$^+$IL17$^+$) cells between mutant and WT mice in either spleen or inguinal lymph nodes. Nor do the proportions of splenic TCRαβ$^+$CD4$^+$CD25$^+$Foxp3$^+$ Treg change when values of EphB2$^{-/-}$ (0.83 ± 0.25), EphB3$^{-/-}$ (0.72 ± 0.16), and WT mice (0.96 ± 0.27) are compared. By contrast, Treg of inguinal lymph nodes show significantly higher values in EphB2$^{-/-}$ (4.29 ± 0.40) and EphB3$^{-/-}$ mice (4.23 ± 0.44) than in WT ones (3.89 ± 0.45).

Recently, we evaluated other lymphoid cell populations particularly those involved in thymocyte selection within the thymus. No differences occurred in either positive selected TCRαβ^{hi}CD4$^+$CD8$^+$CD69$^+$ and TCRαβ^{hi}CD4$^+$CD8$^-$CD69$^+$ thymocytes in both EphB2- (3.00 ± 0.61; 8.00 ± 1.91) and EphB3 (2.17 ± 0.40; 6.60 ± 1.73)-deficient thymi, as compared to WT values (2.47 ± 0.66; 7.09 ± 3.00). Nor did the percentage of both total TCRαβ^{hi}Foxp3$^+$ and TCRαβ^{hi}CD4$^+$Foxp3$^+$ regulatory T cells (Treg) change when EphB2$^{-/-}$ mice (0.94 ± 0.06; 0.69 ± 0.10) and WT ones (0.90 ± 0.18; 0.66 ± 0.12) were compared, although values in EphB3$^{-/-}$ thymi were slightly lower, but not significantly, than in the other two mice analyzed (0.68 ± 0.07; 0.54 ± 0.04).

Negative selection was evaluated in WT and EphB-deficient mice comparing the proportions of total caspase3$^+$CD5$^+$CD69$^+$CD4$^+$ cells and Caspase3$^+$CD5$^+$CD69$^+$CD4$^+$CD8$^+$ cells, as previously proposed (8,31). No significant differences were observed in the proportions of the two cell populations: Caspase3$^+$CD5$^+$CD69$^+$CD4$^+$ (WT: 0.035 ± 0.011; EphB2$^{-/-}$: 0.026 ± 0.008; EphB3$^{-/-}$: 0.035 ± 0.013) and Caspase3$^+$CD5$^+$CD69$^+$CD4$^+$CD8$^+$ (WT: 0.031 ± 0.009; EphB2$^{-/-}$: 0.039 ± 0.008; EphB3$^{-/-}$: 0.020 ± 0.004). Mutant mice living in non-sterile conditions did not show apparently immunological deficits (30) or any signs of autoimmunity since no substantial lymphoid infiltrates occur in their livers or salivary glands (unpublished data).

T-CELL DEVELOPMENT IN AN ALTERED THYMIC EPITHELIUM

Taken together, these results confirm that, except for increased proportions of mutant DN thymocytes and lymph node Treg cells, the percentages of immunocompetent cells do not vary significantly in EphB-deficient animals. Nevertheless, more functional, *in vivo* approaches are necessary to definitively determine the immunological conditions of EphB-deficient animals. By contrast, the TEC network is deeply altered in these mutants making it difficult to explain how these changes do not affect thymocyte development since T–TEC interactions are considered critical for functional maturation of T cells (32, 33).

Results on the effects of Eph/ephrins on thymocyte maturation are few and contradictory presumably reflecting different background of mutant mice, protocols used to evaluate effects of Eph/ephrins and/or specificity of molecules studied, as previously discussed (3). No anomalies have been described in mice with conditionally deleted EphB4 gene in TECs (34), deficiency in EphB6 (24), or in four Ephs, EphB1, B2, B3, and B6 (23). However, other authors have found poor *in vitro* responses of EphB6$^{-/-}$ T cells after anti-CD3 plus anti-CD28 stimulation together as well as *in vivo* decreased hypersensitivity and autoimmune responses (35). On the other hand, the deletion of either ephrin-B1 or ephrin-B2 in thymocytes does not course with thymus phenotypes (36, 37) but the lack of two molecules results in alterations in thymocytes and thymic structure (38), and low sensitivity to different autoimmune models, including experimental autoimmune encephalomyelitis (39) and collagen-induced arthritis (40). We also observed decreased values of positive selected TCRαβ^{hi}CD69$^+$ thymocytes in ephrin-B1 and/or ephrin-B2 deleted in TECs, particularly when using outbred mouse strains. In this case, changes in these lymphoid subsets course with profound alterations in the histological thymus

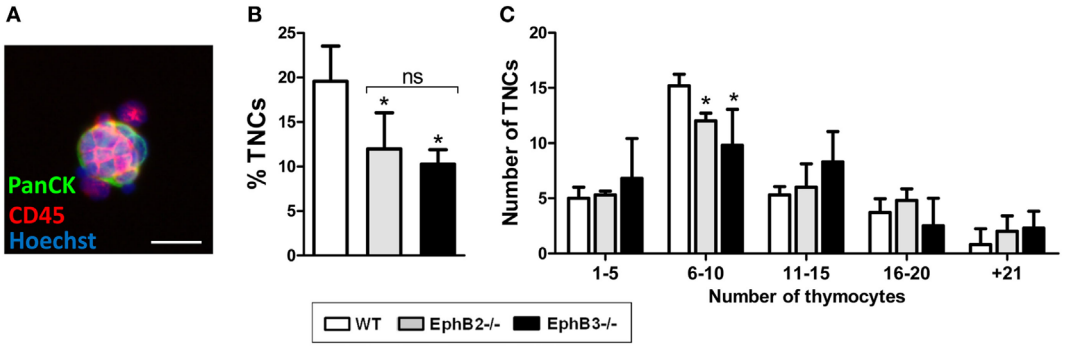

FIGURE 2 | Analysis of thymic nurse complexes (TNCs) in adult WT and EphB-deficient thymi. **(A)** Representative thymic nurse complex formed by thymic epithelial cells, stained with an anti-PanCytokeratin antibody (PanCK, Green) and thymocytes identified by using an anti-CD45 antibody (Red). Nuclei are stained with Hoechst 33342 (Blue). Scale: 20 μm. **(B)** A significantly lower percentage of isolated TNC in EphB2$^{-/-}$ and EphB3$^{-/-}$ thymi than in WT ones. **(C)** According to the number of thymocytes included in the TNCs, six different groups could be established. The figure shows the TNC numbers of WT and EphB-deficient thymi after the analysis of 30 TNCs. In both WT and mutant thymi, the distribution is similar but the frequency of those containing 6–10 thymocytes, which represent the half of total TNC analyzed, is lower in mutant than in WT ones. The significance of the Student's *t*-test probability is indicated as $*p \leq 0.05$. ns: non-significant.

organization (41). In addition, the thymus of EphA4-deficient mice show reduced proportions of both DP TCRαβ^{hi} cells and CD69^{+} cells in correlation with the collapse of thymic cortex (42).

A first glance at these results could suggest a possible association with the pattern of Eph/ephrin expression. At the earliest stages of thymic development, when TEC predominates in T-cell numbers, the proportions of EphB2, EphB3, and ephrin-B2-expressing cells rise to the highest values, sharply declining later, except for ephrin-B2^{+} cells that remain high until E14.5. Furthermore, in these early stages, the proportions of Eph/ephrin B expressing TECs are significantly higher than those of eph/ephrin B^{+} thymocytes (12). It is tentative to speculate that a greater EphB-dependent effect could occur in cell types containing higher numbers of cells expressing these molecules.

Another important point is the condition of thymocyte-TEC contacts in EphB-deficient thymi. Many features of these thymi do not favor the establishment of such interactions. Thus, increased proportions of apoptotic TECs and the appearance of huge epithelial-free areas make difficult thymocyte-TEC contacts in EphB-deficient mice (22). The point is to determine whether these changes are sufficient to provoke severe deficits in the molecular communications between thymocytes and TECs that result in holes in the T-cell repertoire or, by contrast, whether the remaining unchanged epithelial areas expressing key molecules for thymic functional selection, such as β5t, Aire, MHCII, and CD80 are capable of supporting an efficient T-cell maturation. Some recent results relating to the number and cell composition of thymic nurse complexes (TNCs) in EphB-deficient thymi constitute an illustrative example of our hypothesis. Previously, we demonstrated impaired establishment of DP T–TEC conjugates derived from mutant thymi (13).

Thymic nurse complexes were first considered a kind of *ex vivo* specialized thymic microenvironment for T-cell maturation in which a single cTEC constituted lymphostromal complexes with 7–50 thymocytes (43, 44). We analyzed comparatively TNCs (**Figure 2A**) isolated from either WT or EphB-deficient thymi, confirming the expression in them of cTEC (i.e., Ly51, CD205, CD40) markers and MHCII, but not of MTS20 or MTS10 typical molecules of immature cells and mTECs, respectively. Both epithelial cells and thymocytes of nurse complexes also express EphB2, EphB3, and their ligands, ephrin-B1 and ephrin-B2, but the number of complexes yielded from mutant thymi was significantly lower than those from WT ones (**Figure 2B**). Most isolated TNCs contained 6–10 thymocytes and those composed of more than 21 thymocytes were the least represented. Mutant TNCs showed a similar range but exhibited significant reduced numbers of the most frequent ones containing 6–10 thymocytes (**Figure 2C**), suggesting that the lack of Eph/ephrin B affected the T–TEC interactions necessary to form the TNCs.

Although it is currently recognized that nurse complexes are special cortical areas involved in positive selection where long-lived DP thymocytes undergo secondary TCRα chain rearrangements (45), we failed to find changes in the proportions of positively selected TCRαβ^{hi}CD69^{+} thymocytes indirectly suggesting that alterations found in TNCs from EphB-deficient thymi are not sufficient to impair positive selection.

These results demonstrate therefore that mutant TECs express all the molecules necessary to interact with developing thymocytes and to promote their proper differentiation, although their appearance and maturation is delayed with respect to the WT epithelium (12, Montero-Herradón et al 2017, submitted manuscript)[1]. In this respect, it has been claimed that just a few unaltered areas of thymic stroma could be sufficient to support a quite normal T-cell development (21, 46). Conditional deletion of Stat3 in K5^{+} TEC that courses with changes in medulla histology and decreased proportions of mature MHCII^{hi}Aire^{+} mTECs does not affect autoimmune reactivity (47). More recently, specific deletion in TECs of the LTβR gene produces important changes in mTECs, including the disruption of typical 3D medulla organization with small, scattered medulla foci, and reduced numbers of mTECs^{lo}, mTECs^{hi}, and Aire cells (8), quite similar to the phenotype described for the thymic medulla of EphB-deficient mice. However, the frequencies of CD4^{+} and CD8^{+} thymocytes

are unchanged, and the peripheral lymphoid organs of these mice are intact, suggesting that they do not undergo autoimmunity or exhibit an altered T-cell repertoire.

In agreement with our hypothesis, these authors conclude that despite limited numbers of tissue-related antigen-producing cells, the capacity of these mutant thymi for self-antigen production dependent on mTECs would exceed the threshold required for tolerance induction (8), explaining the absence of immune deficits.

ETHICS STATEMENT

The study was carried out in accordance with the recommendations of the "Ethic Committee for Animal Research" of Complutense University. The protocols were approved by the Regional Government of Madrid.

AUTHOR CONTRIBUTIONS

JM, JG, and AZ have contributed to the design of the manuscript. SM, BS, and DA have provided technical results. All authors have read and accepted the final manuscript.

FUNDING

This study was supported by the Spanish Ministry of Economy and Competitiveness under grant BFU2013-41112-R and Cell Therapy Network (RD12/0019/0007 and RD16/0011/0002).

REFERENCES

1. Perez White BE, Getsios S. Eph receptor and ephrin function in breast, gut, and skin epithelia. *Cell Adh Migr* (2014) 8:327–38. doi:10.4161/19336918.2014.970012
2. Wilkinson DG. Regulation of cell differentiation by Eph receptor and ephrin signaling. *Cell Adh Migr* (2014) 8:339–48. doi:10.4161/19336918.2014.970007
3. Garcia-Ceca J, Alfaro D, Montero-Herradon S, Tobajas E, Munoz JJ, Zapata AG. Eph/Ephrins-mediated thymocyte–thymic epithelial cell interactions control numerous processes of thymus biology. *Front Immunol* (2015) 6:333. doi:10.3389/fimmu.2015.00333
4. Knoll B, Drescher U. Ephrin-As as receptors in topographic projections. *Trends Neurosci* (2002) 25:145–9. doi:10.1016/S0166-2236(00)02093-2
5. Mentlein R, Kendall MD. The brain and thymus have much in common: a functional analysis of their microenvironments. *Immunol Today* (2000) 21:133–40. doi:10.1016/S0167-5699(99)01557-1
6. Stimamiglio MA, Jimenez E, Silva-Barbosa SD, Alfaro D, Garcia-Ceca JJ, Munoz JJ, et al. EphB2-mediated interactions are essential for proper migration of T cell progenitors during fetal thymus colonization. *J Leukoc Biol* (2010) 88:483–94. doi:10.1189/jlb.0210079
7. Munoz JJ, Cejalvo T, Alonso-Colmenar LM, Alfaro D, Garcia-Ceca J, Zapata A. Eph/Ephrin-mediated interactions in the thymus. *Neuroimmunomodulation* (2011) 18:271–80. doi:10.1159/000329490
8. Cosway EJ, Lucas B, James KD, Parnell SM, Carvalho-Gaspar M, White AJ, et al. Redefining thymus medulla specialization for central tolerance. *J Exp Med* (2017) 214:3183–95. doi:10.1084/jem.20171000
9. Alfaro D, Munoz JJ, Garcia-Ceca J, Cejalvo T, Jimenez E, Zapata A. Alterations in the thymocyte phenotype of EphB-deficient mice largely affect the double negative cell compartment. *Immunology* (2008) 125:131–43. doi:10.1111/j.1365-2567.2008.02828.x
10. Montero-Herradon S, Garcia-Ceca J, Sanchez Del Collado B, Alfaro D, Zapata AG. Eph/ephrin-B-mediated cell-to-cell interactions govern MTS20(+) thymic epithelial cell development. *Histochem Cell Biol* (2016) 146:167–82. doi:10.1007/s00418-016-1431-x
11. Garcia-Ceca J, Jimenez E, Alfaro D, Cejalvo T, Chumley MJ, Henkemeyer M, et al. On the role of Eph signalling in thymus histogenesis; EphB2/B3 and the organizing of the thymic epithelial network. *Int J Dev Biol* (2009) 53:971–82. doi:10.1387/ijdb.082702jg
12. Montero-Herradon S, Garcia-Ceca J, Zapata AG. EphB receptors, mainly EphB3, contribute to the proper development of cortical thymic epithelial cells. *Organogenesis* (2017) 13:192–211. doi:10.1080/15476278.2017.1389368
13. Alfaro D, Garcia-Ceca JJ, Cejalvo T, Jimenez E, Jenkinson EJ, Anderson G, et al. EphrinB1-EphB signaling regulates thymocyte–epithelium interactions involved in functional T cell development. *Eur J Immunol* (2007) 37:2596–605. doi:10.1002/eji.200737097
14. Yu G, Mao J, Wu Y, Luo H, Wu J. Ephrin-B1 is critical in T-cell development. *J Biol Chem* (2006) 281:10222–9. doi:10.1074/jbc.M510320200
15. Alfaro D, Garcia-Ceca J, Farias-de-Oliveira DA, Terra-Granado E, Montero-Herradon S, Cotta-de-Almeida V, et al. EphB2 and EphB3 play an important role in the lymphoid seeding of murine adult thymus. *J Leukoc Biol* (2015) 98:883–96. doi:10.1189/jlb.1HI1114-568R
16. Luo H, Wu Z, Qi S, Jin W, Han B, Wu J. Ephrinb1 and Ephrinb2 are associated with interleukin-7 receptor alpha and retard its internalization from the cell surface. *J Biol Chem* (2011) 286:44976–87. doi:10.1074/jbc.M111.316414
17. Ceredig R, Rolink T. A positive look at double-negative thymocytes. *Nat Rev Immunol* (2002) 2:888–97. doi:10.1038/nri937
18. Hirano K, Negishi N, Yazawa M, Yagita H, Habu S, Hozumi K. Delta-like 4-mediated Notch signaling is required for early T-cell development in a three-dimensional thymic structure. *Eur J Immunol* (2015) 45:2252–62. doi:10.1002/eji.201445123
19. Garcia-Ceca J, Alfaro D, Montero-Herradon S, Zapata AG. Eph/ephrinB signalling is involved in the survival of thymic epithelial cells. *Immunol Cell Biol* (2013) 91:130–8. doi:10.1038/icb.2012.59
20. Jenkinson WE, Jenkinson EJ, Anderson G. Differential requirement for mesenchyme in the proliferation and maturation of thymic epithelial progenitors. *J Exp Med* (2003) 198:325–32. doi:10.1084/jem.20022135
21. Revest JM, Suniara RK, Kerr K, Owen JJ, Dickson C. Development of the thymus requires signaling through the fibroblast growth factor receptor R2-IIIb. *J Immunol* (2001) 167:1954–61. doi:10.4049/jimmunol.167.4.1954
22. Garcia-Ceca J, Montero-Herradon S, Alfaro D, Zapata AG. Increased epithelial-free areas in thymuses with altered EphB-mediated thymocyte–thymic epithelial cell interactions. *Histochem Cell Biol* (2017) 148:381–94. doi:10.1007/s00418-017-1583-3
23. Kawano H, Katayama Y, Minagawa K, Shimoyama M, Henkemeyer M, Matsui TA. novel feedback mechanism by Ephrin-B1/B2 in T-cell activation involves a concentration-dependent switch from costimulation to inhibition. *Eur J Immunol* (2012) 42:1562–72. doi:10.1002/eji.201142175
24. Shimoyama M, Matsuoka H, Nagata A, Iwata N, Tamekane A, Okamura A, et al. Developmental expression of EphB6 in the thymus: lessons from EphB6 knockout mice. *Biochem Biophys Res Commun* (2002) 298:87–94. doi:10.1016/S0006-291X(02)02399-9
25. Noren NK, Yang NY, Silldorff M, Mutyala R, Pasquale EB. Ephrin-independent regulation of cell substrate adhesion by the EphB4 receptor. *Biochem J* (2009) 422:433–42. doi:10.1042/BJ20090014
26. Henkemeyer M, Itkis OS, Ngo M, Hickmott PW, Ethell IM. Multiple EphB receptor tyrosine kinases shape dendritic spines in the hippocampus. *J Cell Biol* (2003) 163:1313–26. doi:10.1083/jcb.200306033
27. Foo SS, Turner CJ, Adams S, Compagni A, Aubyn D, Kogata N, et al. Ephrin-B2 controls cell motility and adhesion during blood-vessel-wall assembly. *Cell* (2006) 124:161–73. doi:10.1016/j.cell.2005.10.034
28. Arthur A, Zannettino A, Panagopoulos R, Koblar SA, Sims NA, Stylianou C, et al. EphB/ephrin-B interactions mediate human MSC attachment, migration and osteochondral differentiation. *Bone* (2011) 48:533–42. doi:10.1016/j.bone.2010.10.180
29. Rodewald HR, Paul S, Haller C, Bluethmann H, Blum C. Thymus medulla consisting of epithelial islets each derived from a single progenitor. *Nature* (2001) 414:763–68. doi:10.1038/414763a
30. Munoz JJ, Garcia-Ceca J, Alfaro D, Cejalvo T, Stimamiglio MA, Jimenez E, et al. Eph and ephrin: key molecules for the organization and function of the thymus gland. *Inmunología* (2009) 28:19–31. doi:10.1016/S0213-9626(09)70024-1

31. Stritesky GL, Xing Y, Erickson JR, Kalekar LA, Wang X, Mueller DL, et al. Murine thymic selection quantified using a unique method to capture deleted T cells. *Proc Natl Acad Sci U S A* (2013) 110:4679–84. doi:10.1073/pnas.1217532110
32. Anderson G, Takahama Y. Thymic epithelial cells: working class heroes for T cell development and repertoire selection. *Trends Immunol* (2012) 33:256–63. doi:10.1016/j.it.2012.03.005
33. Takahama Y, Ohigashi I, Baik S, Anderson G. Generation of diversity in thymic epithelial cells. *Nat Rev Immunol* (2017) 17:295–305. doi:10.1038/nri.2017.12
34. Jin W, Luo H, Wu J. Effect of reduced EPHB4 expression in thymic epithelial cells on thymocyte development and peripheral T cell function. *Mol Immunol* (2014) 58:1–9. doi:10.1016/j.molimm.2013.10.008
35. Luo H, Yu G, Tremblay J, Wu J. EphB6-null mutation results in compromised T cell function. *J Clin Invest* (2004) 114:1762–73. doi:10.1172/JCI21846
36. Jin W, Qi S, Luo H. The effect of conditional EFNB1 deletion in the T cell compartment on T cell development and function. *BMC Immunol* (2011) 12:68. doi:10.1186/1471-2172-12-68
37. Jin W, Qi S, Luo H. T cell-specific deletion of EFNB2 minimally affects T cell development and function. *Mol Immunol* (2012) 52:141–7. doi:10.1016/j.molimm.2012.05.008
38. Luo H, Charpentier T, Wang X, Qi S, Han B, Wu T, et al. Efnb1 and Efnb2 proteins regulate thymocyte development, peripheral T cell differentiation, and antiviral immune responses and are essential for interleukin-6 (IL-6) signaling. *J Biol Chem* (2011) 286:41135–52. doi:10.1074/jbc.M111.302596
39. Luo H, Broux B, Wang X, Hu Y, Ghannam S, Jin W, et al. EphrinB1 and EphrinB2 regulate T cell chemotaxis and migration in experimental autoimmune encephalomyelitis and multiple sclerosis. *Neurobiol Dis* (2016) 91:292–306. doi:10.1016/j.nbd.2016.03.013
40. Hu Y, Wang X, Wu Y, Jin W, Cheng B, Fang X, et al. Role of EFNB1 and EFNB2 in mouse collagen-induced arthritis and human rheumatoid arthritis. *Arthritis Rheumatol* (2015) 67:1778–88. doi:10.1002/art.39116
41. Cejalvo T, Munoz JJ, Tobajas E, Fanlo L, Alfaro D, Garcia-Ceca J, et al. Ephrin-B-dependent thymic epithelial cell–thymocyte interactions are necessary for correct T cell differentiation and thymus histology organization: relevance for thymic cortex development. *J Immunol* (2013) 190:2670–81. doi:10.4049/jimmunol.1201931
42. Munoz JJ, Alfaro D, Garcia-Ceca J, Alonso CL, Jimenez E, Zapata A. Thymic alterations in EphA4-deficient mice. *J Immunol* (2006) 177:804–13. doi:10.4049/jimmunol.177.2.804
43. Chilukuri RV, Patel VK, Martinez M, Guyden JC, Samms MD. The antigenic determinant that defines thymic nurse cells is expressed by thymic epithelial progenitor cells. *Front Cell Dev Biol* (2014) 2(13):1–6. doi:10.3389/fcell.2014.00013
44. Hendrix TM, Chilukuri RV, Martinez M, Olushoga Z, Blake A, Brohi M, et al. Thymic nurse cells exhibit epithelial progenitor phenotype and create unique extra-cytoplasmic membrane space for thymocyte selection. *Cell Immunol* (2010) 261:81–92. doi:10.1016/j.cellimm.2009.11.004
45. Nakagawa Y, Ohigashi I, Nitta T, Sakata M, Tanaka K, Murata S, et al. Thymic nurse cells provide microenvironment for secondary T cell receptor alpha rearrangement in cortical thymocytes. *Proc Natl Acad Sci U S A* (2012) 109:20572–7. doi:10.1073/pnas.1213069109
46. Osada M, Ito E, Fermin HA, Vazquez-Cintron E, Venkatesh T, Friedel RH, et al. The Wnt signaling antagonist Kremen1 is required for development of thymic architecture. *Clin Dev Immunol* (2006) 13:299–319. doi:10.1080/17402520600935097
47. Lomada D, Jain M, Bolner M, Reeh KA, Kang R, Reddy MC, et al. Stat3 signaling promotes survival and maintenance of medullary thymic epithelial cells. *PLoS Genet* (2016) 12:e1005777. doi:10.1371/journal.pgen.1005777

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

MINI REVIEW
published: 12 July 2018
doi: 10.3389/fendo.2018.00381

Abnormal T-Cell Development in the Thymus of Non-obese Diabetic Mice: Possible Relationship With the Pathogenesis of Type 1 Autoimmune Diabetes

Daniella A. Mendes-da-Cruz[1,2]*, *Julia P. Lemos*[1,2], *Geraldo A. Passos*[3,4] and *Wilson Savino*[1,2]*

[1] Laboratory on Thymus Research, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, [2] National Institute of Science and Technology on Neuroimmunomodulation, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, [3] Department of Morphology, Physiology and Basic Pathology, Ribeirão Preto Medical School, School of Dentistry of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil, [4] Department of Genetics, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, Brazil

OPEN ACCESS

Edited by:
Vincent Geenen,
University of Liège, Belgium

Reviewed by:
Karsten Kretschmer,
Technische Universität Dresden, Germany
Nils Lambrecht,
VA Long Beach Healthcare System and University of California, Irvine, United States

***Correspondence:**
Daniella A. Mendes-da-Cruz
daniella@ioc.fiocruz.br
Wilson Savino
savino.w@gmail.com

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: 31 January 2018
Accepted: 22 June 2018
Published: 12 July 2018

Citation:
Mendes-da-Cruz DA, Lemos JP, Passos GA and Savino W (2018) Abnormal T-Cell Development in the Thymus of Non-obese Diabetic Mice: Possible Relationship With the Pathogenesis of Type 1 Autoimmune Diabetes. Front. Endocrinol. 9:381. doi: 10.3389/fendo.2018.00381

Type 1 diabetes (T1D) is an autoimmune disease caused by the destruction of insulin-producing cells in the pancreas, by direct interactions with autoreactive pancreas infiltrating T lymphocytes (PILs). One of the most important animal models for this disease is the non-obese diabetic (NOD) mouse. Alterations in the NOD mouse thymus during the pathogenesis of the disease have been reported. From the initial migratory disturbances to the accumulation of mature thymocytes, including regulatory Foxp3$^+$ T cells, important mechanisms seem to regulate the repertoire of T cells that leave the thymus to settle in peripheral lymphoid organs. A significant modulation of the expression of extracellular matrix and soluble chemoattractant molecules, in addition to integrins and chemokine receptors, may contribute to the progressive accumulation of mature thymocytes and consequent formation of giant perivascular spaces (PVS) that are observed in the NOD mouse thymus. Comparative large-scale transcriptional expression and network analyses involving mRNAs and miRNAs of thymocytes, peripheral T CD3$^+$ cells and PILs provided evidence that in PILs chemokine receptors and mRNAs are post-transcriptionally regulated by miR-202-3p resulting in decreased activity of these molecules during the onset of T1D in NOD mice. In this review, we discuss the abnormal T-cell development in NOD mice in the context of intrathymic expression of different migration-related molecules, peptides belonging to the family of insulin and insulin-like growth factors as well as the participation of miRNAs as post-transcriptional regulators and their possible influence on the onset of aggressive autoimmunity during the pathogenesis of T1D.

Keywords: non-obese diabetic mouse, type 1 diabetes, thymus, autoimmune diabetes, insulin, insulin-like growth factor, miRNA

INTRODUCTION

Type 1 diabetes (T1D) is a multifactorial disease caused by autoimmune destruction of pancreatic beta cells, which results in a breakdown of insulin production and glucose metabolism (1). The mechanisms involved in autoimmunity during the pathogenesis of T1D include factors of humoral immunity, such as the presence of circulating autoantibodies (anti-insulin, among other islet autoantibodies), that can be used as biomarkers of the disease (2–4). Mechanisms involving cellular immunity are evidenced by the presence of mononuclear cell infiltrates in the islets of Langerhans. CD8 T cells are the most predominant infiltrating-cells, followed by macrophages, CD4 T cells, B lymphocytes and plasma cells (5). In addition to the cellular infiltrate, the upregulation of MHC class I on β-cells may increase their susceptibility to T-cell–mediated killing (6). Most of the studies in humans were performed in pancreas samples removed post-mortem. Due to the limited availability concerning the samples and difficulties in studying the mechanisms of autoimmunity in humans, the use of experimental models are essential for studies on the pathogenesis of T1D. Among the available experimental models, the non-obese diabetic (NOD) mouse is particularly well characterized. They spontaneously develop the disease and present several characteristics that are similar to the pathogenesis of the human T1D (7, 8). Briefly, insulitis starts in general at 3 weeks of age in female mice, concomitantly with the appearance of initial thymic alterations, and the disease onset occurs at 10–12 weeks, depending on the colony. At this point, different alterations in the thymus have been described, as we discuss below. Nevertheless, before entering this discussion, it seems worthwhile to provide a basic background on some physiological aspects of the thymus, including the intrathymic T-cell differentiation, as well as production of hormones by thymic cells.

THE THYMUS AND THYMOCYTE DEVELOPMENT

The thymus is a primary lymphoid organ where T cells are generated. Inside the thymic tissue, precursor cells pass through distinct differentiation stages until becoming mature CD4 or CD8 single-positive (SP) thymocytes expressing the T-cell receptor (TCR), which are ready to emigrate to peripheral lymphoid organs and properly finish their maturation (9). Cell differentiation occurs in parallel with cell migration, so that the immature double-negative (DN) for the $CD4^{-}CD8^{-}$ co-receptors and double-positive (DP) $CD4^{+}CD8^{+}$ thymocytes are localized in the cortical region of the thymic lobules, while more mature CD4SP or CD8SP thymocytes are localized in the medulla (10). DP thymocytes express low amounts of TCR after gene rearrangement. This expression is increased during differentiation to TCR^{high} CD4SP or CD8SP cells. Differentiating cells undergo apoptosis if their TCR interact with high avidity with self-antigens coupled to major histocompatibility complex (MHC) class I or class II molecules expressed by microenvironmental cells in the thymus, in a process called negative selection. Alternatively, some clones that recognize self-antigens with high avidity become regulatory $CD4^{+}CD25^{+}Foxp3^{+}$ T cells (Treg), a mechanism that seems to depend on TCR signaling avidity and duration, TGF-β-mediated survival and cytokines, such as IL-2, IL-7, and IL-15 (11, 12). These thymus-derived Treg cells account for the majority of Tregs in the periphery, compared with Tregs differentiated from conventional naïve T cells (13). Together, these processes avoid the development of self-antigen reactive cells and therefore prevents autoimmunity (14).

In the thymus, the expression of many peripheral tissue antigens (PTAs) in medullary thymic epithelial cells (mTEC) is regulated by the autoimmune regulator (AIRE) transcription factor (15). The PTAs are presented by MHC molecules and can induce negative selection of autoreactive thymocytes (16). The homozygous loss or mutations in the Aire gene cause the autoimune polyendocrinopathy–candidosis–ectodermal dystrophy (APECED) syndrome in humans, characterized by the development of autoimmune diseases including T1D in 10–20% of the cases (17–19). In mice, Aire disruption leads to immune cell infiltration in several organs and APECED-autoimmune like manifestations (15, 20).

While migrating through the thymic lobules, developing thymocytes also interact with other microenvironmental cells such as dendritic cells (DCs) and macrophages, as well as with extracellular matrix (ECM) molecules and soluble proteins such as cytokines, chemokines, growth factors and thymic hormones (thymulin and thymopoietin, for example). Other hormones produced by endocrine glands (growth hormone, glucocorticoids, prolactin, oxytocin and insulin) can also be produced locally, and play a role in the physiology of the thymus and the generation of the T-cell repertoire (10).

INTRATHYMIC EXPRESSION OF PEPTIDES AND RECEPTORS FROM THE INSULIN/IGF FAMILY

Insulin is a polypeptidic hormone produced as a pre-prohormone, the pre-proinsulin, which is processed to proinsulin that is cleaved, in turn, to mature insulin. Only pancreatic beta cells are capable to secrete mature insulin in response to glucose (21). Despite that, proinsulin gene is naturally expressed at low levels in fetal and postnatal thymi in humans, rats and mice (22). Although the expression of proinsulin in the thymus is not necessary for T cell differentiation and growth (23, 24), variations in the expression of the insulin gene in the thymus, but not in the pancreas, in both humans and mice, can modulate self-tolerance to insulin, with the expression levels being inversely correlated with T1D susceptibility (21, 25).

In humans, the insulin gene is under the control of a variable number of tandem repeats (VNTR) minisatellites, mapping 5′ to the insulin gene promoter. VNTR, commonly known as IDDM2 susceptibility locus, are extremely polymorphic regions both in size and sequence (25, 26), and three allele classes have been characterized: class I, composed by 20–63 repeats of the consensus unit ACAGGGGTCTGGGG, class II alleles containing

64–139 repeats and class III alleles containing 140 to >200 repeats. Although VNTR have little effect in pancreatic insulin transcription, class I alleles in the thymus correlate with low and class III with high levels of insulin mRNA (25, 27).

There is no VNTR regions in mice, but two nonallelic insulin genes Ins1 and Ins2 encoding proinsulin 1 and 2 respectively (28, 29). Both insulin genes are expressed by 1–3% of mTECs in the thymus under control of AIRE (15, 30, 31), but Ins2 expression is predominant in the thymus. Although suggested that this predominant expression leads to a higher tolerance to proinsulin 2 (32), it was demonstrated that proinsulin 2 expression leads to T cell tolerance to an epitope shared by both proinsulin 1 and 2 (33). The copy numbers of insulin gene in the mouse thymus inversely correlates to the numbers of insulin-specific autoreactive T cells in the periphery, so that mice expressing low levels of thymic insulin, (even though pancreatic insulin remains unaltered), present peripheral reactivity to insulin, whereas mice with normal thymic insulin expression have no significant response (34). This effect is transferable by thymic transplantation (35), showing that thymic insulin expression plays a critical role in thymic selection and T1D susceptibility.

Insulin-like growth factors (IGF) 1 and 2 are polypeptidic growth factors, members of the family of insulin-related peptides, produced in many tissues where they can play endocrine and paracrine functions (36). Both IGF-1 and 2 can bind to type 1 and 2 IGF receptors (IGF-1R/IGF-2R) with high affinity and to insulin receptors (INS-R), with low affinity (37). All the genes of the insulin family are expressed in the thymus during the fetal life; IGF2 is predominantly expressed in the rat, mouse and human thymi by TEC and Thymic Nurse Complexes (TNC), followed by IGF1, expressed by TECs and macrophages. The proinsulin genes are expressed by mTECs and DCs (38–41). In general, protein levels are related with gene levels in the case of these molecules.

After birth, IGF-2 gene expression and protein levels decrease and reach the same levels of IGF-1 (32). IGF-2 participates both in T cell development and negative selection (42). Studies using fetal thymic organ cultures (FTOC) demonstrated that the blockage of IGF-mediated signaling between TEC and thymocytes inhibits early T cell proliferation and differentiation (23). Specific anti-IGF-1 antibodies treatment lead to a decreased DN relative cell numbers while the inhibition of IGF-2, IGF-1R or IGF-2R impaired differentiation from the DN to the DP stage. The same study showed a decrease in total T cell numbers under treatment with anti-IGF-1R and anti-IGF-2R antibodies. Moreover, transgenic IGF-2 expression resulted in abnormalities in the terminal differentiation and increased proliferation of TECs. The deposition of fibronectin and laminin is enhanced in human TEC cultures and in the thymus of IGF-2 transgenic mice, in parallel with the enhancement of thymocyte adhesion to TEC monolayers and thymocyte migration (43).

IGF-2 expression by the thymic epithelium is under control of AIRE, and the IGF-2 gene is located adjacent to the Ins gene (44, 45). Its predominant expression among insulin family members could be explained by IGF-2 close homology to the other members with high conserved peptides sequences of the family. This could lead to the development of tolerance to IGF-2 and related molecules, including insulin (45). Igf-2$^{-/-}$ mice present weaker tolerance to insulin when compared with wild type animals and the production of specific antibodies to IGF-2 is more difficult than to IGF-1 or insulin (46–48).

IGF-1 and its receptor are implicated in several growth hormone (GH) effects in the thymus, as TEC proliferation and thymocyte/TEC adhesion (49), as we further discuss below.

GH/IGF-1 AXIS IN THE THYMUS

Growth hormone is a member of a family of growth factors that includes prolactin and other hormones. It is produced and stored mainly in the anterior pituitary under control of hypothalamic hormones, as the GH-releasing hormone, hypothalamic GH release-inhibiting factor and somatostatin (50), although the production by other cell types was observed, including leukocytes and TECs (51). The early experiments showing that GH is thymotropic revealed that GH-deficient mice present thymus atrophy and this effect is also observed after GH anti-serum treatment of mice with intact pituitary (52).

The GH receptor (GHR) is expressed in cortical and medullary TECs (53, 54) as well as in thymocytes (51, 55), and plays a role in thymic function and T-cell differentiation. The decline of GH production is related with thymic involution (56). Moreover, transgenic mice overexpressing GH have an enlarged thymus, as well as mice and humans treated with recombinant forms of the hormone (57). GH can also modulate the thymic microenvironment by increasing the secretion of cytokines, chemokines and thymulin, consequently modulating thymocyte adhesion and migration (57–60).

As mentioned above, some of the GH effects in the thymus are mediated by IGF-1. Murine TEC lines treated with GH or IGF-1, present an enhancement in ECM molecules production as type IV collagen, fibronectin and laminin, besides the expression of the integrins VLA-5 (alpha 5 beta 1 integrin, a fibronectin receptor) and VLA-6 (alpha 6 beta 1 integrin, a laminin receptor). Treatment with GH also augmented the thymocyte/TEC adhesion, a phenomenon that was blocked by anti-IGF-1 and anti-IGF-1R antibodies (61).

Since the interactions of thymocyte and TECs are crucial for thymocyte development and thymus physiology, one can argue that together, the GH/IGF-1 axis, besides IGF-2 and insulin can shape the T-cell repertoire. Moreover, it is conceivable that defects in the negative selection against PTAs related to this family might cause autoimmunity, as for example T1D, in the case of insulin-related peptides expressed intrathymically.

THYMIC ALTERATIONS IN NOD MICE

Several morphological and phenotypic alterations are observed in the NOD mouse thymus. The most evident is the formation of giant perivascular spaces (PVSs), which are filled with mature CD4SP and CD8SP cells, B cells and regulatory Foxp3^{+} cells (62–64). We have described that cells inside giant PVSs present

TABLE 1 | Alterations observed in the NOD mouse thymus.

	Alteration*	Affected compartments	References
THYMIC PARENCHYMA STRUCTURES			
PVS	↑↑↑	Medullary region	(62)
TECs	↓	Medullary region	(62)
CELL MIGRATION-RELATED MOLECULES			
VLA-5	↓↓↓	Mainly CD4SP CD8SP and Foxp3^{+} regulatory T cells	(64, 65)
VLA-4	↑	DP, CD4SP and CD8SP thymocytes	(64, 65)
VLA-6	↑↑↑	All thymocyte subpopulations	(64)
CXCR4	↓	CD8SP thymocytes	(64)
CXCL12	↑	Mainly inside giant PVSs	(64)
Fibronectin	↑	Mainly inside giant PVSs	(64, 65)
Laminin	↑	Mainly inside giant PVSs	(64, 65)
Type I and IV collagens	↑	Mainly inside giant PVSs	(62)
INSULIN FAMILY-RELATED PEPTIDES AND RECEPTORS			
Insulin	↓↓	mTECs	(66)
IGF-1, IGF-2, INS-R, IGF-1R, IGF-2R	ND	–	–
GH, GHR	ND	–	–
miRNAs			
miR-19a	↓/–	Thymocytes TCR^{+}/NKT17	(67)
miR-19b	↓/–	Thymocytes TCR^{+}/NKT17	(67)
miR-133b	–/↑↑	Thymocytes TCR^{+}/NKT17	(67)
miR-124a	↑/–	Thymocytes TCR^{+}/NKT17	(67)
miR-326	↓/–	Thymocytes TCR^{+}/NKT17	(67)

**Alteration comparing NOD with other inbred mouse strains; PVS, perivascular space; TEC, thymic epithelial cell; IGF, insulin-like growth factor; INS-R, insulin receptor; IGF-R, insulin-like growth factor receptor; GH, growth hormone; GHR, growth hormone receptor; NKT17, IL-17-producing natural killer T cells; ND, not described.*

a defect in the membrane expression of the integrin-type fibronectin receptor VLA-5 (CD49e/CD29) that may lead to their accumulation and retention in the thymus (65). The formation of giant PVSs also changes the TEC network and ECM contents, both inside PVS and in the thymic parenchyma. Particularly, there is an important deposition of fibronectin inside these spaces (**Table 1**).

The accumulation of thymocytes and enlargement of PVS starts to be observed at 4 weeks of age in female mice, which are more susceptible for T1D. Clear-cut giant PVS are observed in pre-diabetic mice (9–12 weeks of age), that already present insulitis (62).

Ex vivo functional assays revealed that NOD thymocytes have a defect in the migratory capacity toward fibronectin, but not laminin. Interestingly, migration toward the chemokine CXCL12 is enhanced, and a synergic effect is observed when CXCL12 is combined with ECM molecules. In the case of fibronectin combined with CXCL12, despite the synergic effect, migration of NOD thymocytes is reduced compared with controls (64, 65).

Another experimental strategy trying to understand the role of VLA-5 in thymocyte accumulation in giant PVS in NOD mice comprised ECM-transmigration assays, which mimic the migration of thymocytes through fibronectin-enriched PVSs and then the transmigration through endothelium. These experiments revealed that NOD thymocytes that first encounter fibronectin molecules transmigrate less then controls (64). Conversely, differences in transmigration assays were not observed when laminin was applied, reinforcing the concept that VLA-5/fibronectin interactions can play a role in the accumulation of thymocytes in PVS during the pathogenesis of T1D (**Figure 1**).

As mentioned above, thymic insulin expression plays a role in thymic selection processes and T1D development. The expression levels of insulin genes are also altered in the NOD mouse thymus. The Ins2 gene expression is normal at 2 weeks of age but become lower at 3 weeks, which may favor loss of tolerance to insulin in NOD mice (66, 68). Moreover, Ins2$^{-/-}$ NOD mice have accelerated insulitis and autoimmune diabetes onset in females, increased disease in males, with enhanced prevalence of insulin autoantibodies and stronger insulin response (33). Conversely, insulitis and diabetes onset were delayed in NOD Isn1$^{-/-}$ mice, which can be explained by the dominance of the Ins2 gene in the thymus, whereas Ins1 is more prominent in pancreatic beta cells (69). Prevention of both insulitis and diabetes can be seen in transgenic NOD mice expressing increased levels of Ins2 under the MHC class II promoter (70), and also after intrathymic administration of insulin (71).

The expansion of autoreactive T cell clones in NOD mice can also be affected by proinsulin gene expression. Although the numbers of CD4SP and CD8SP thymocytes do not change, proinsulin-1 or−2 deficiency in NOD mice causes changes in the T-cell repertoire generated in the thymus and peripheral lymphoid organs, and is associated with a significant expansion of insulin–reactive CD8SP T cell clones in the pancreatic draining lymph node (72).

The effects of IGF-1 on cell trafficking were also analyzed in the adoptive T cell transfer model in NOD mice (73). T cells from diabetic NOD Thy-1.2 mice were injected into congenic NOD Thy-1.1 mice. In this model, reconstitution of the thymus of irradiated recipients with donor cells was not influenced by IGF-1 treatment, but the percentage of donor T cells was significantly reduced in the spleen of IGF-1 treated mice in contrast to the thymus, suggesting that IGF-1 could influence T cell trafficking from the thymus to peripheral lymphoid organs. This might be due to effects of IGF-1 upon the Sphingosine kinase/sphingosine-1-phosphate axis, as demonstrated for myoblast differentiation (74).

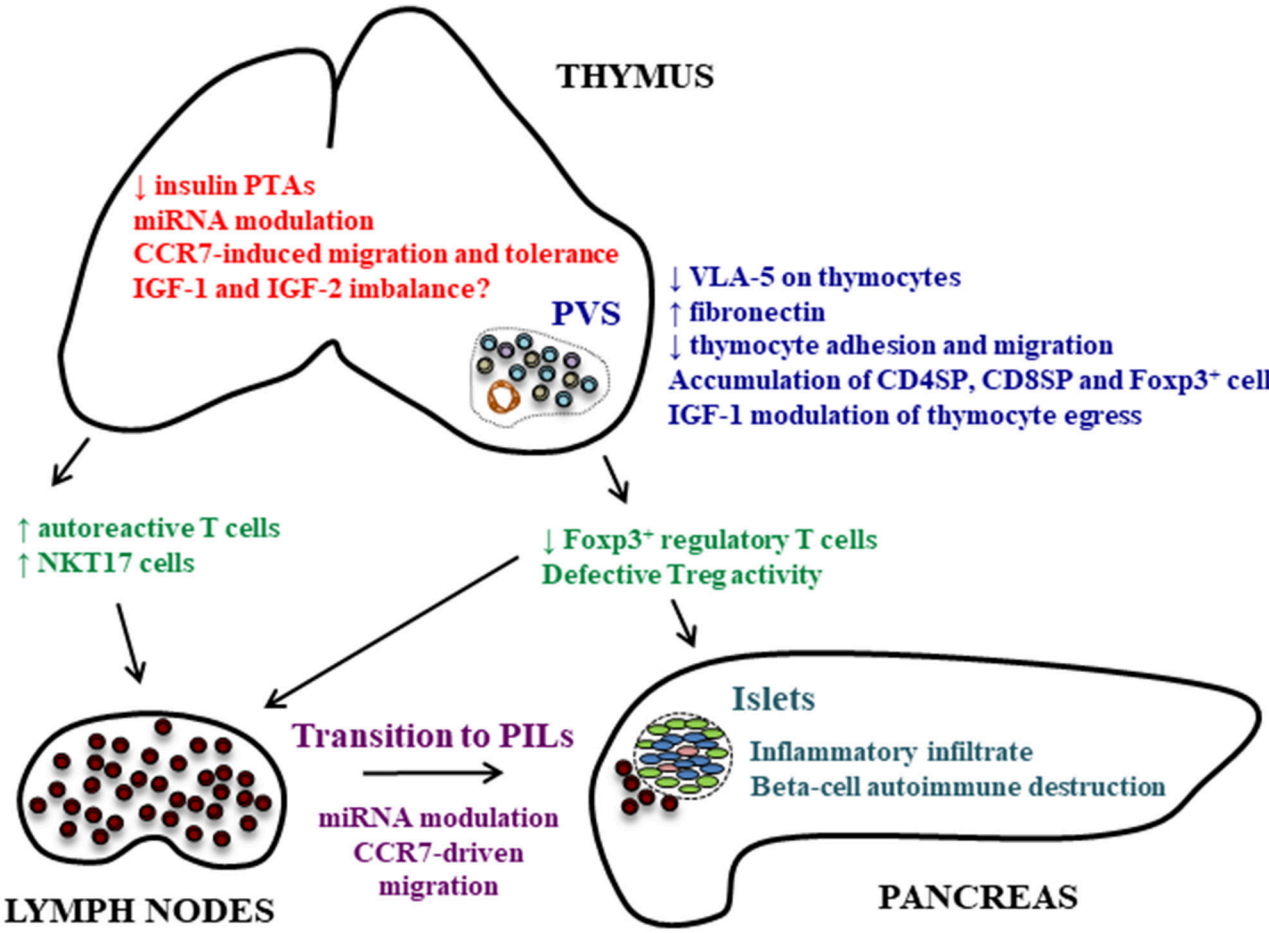

FIGURE 1 | Thymic alterations that can play a role in autoimmune pathogenesis of T1D. Different thymic alterations are observed in the NOD mouse thymus concerning the expression and role of molecules involved in cell adhesion and migration, peptidic hormones under control of the AIRE gene and miRNAs. The diminished insulin expression in the thymus and miRNA modulation of PTAs can lead to the generation of autoreactive cells. The defect in VLA-5 membrane expression on thymocytes and modulation of chemokine receptors are related with the accumulation of thymocytes, including Foxp3^{+} regulatory cells, and the formation of fibronectin-enriched giant PVSs. This accumulation of thymocytes may also be modulated by IGF-1, and can explain the reduced Treg numbers in peripheral lymphoid organs and pancreas affecting the balance between Tregs and effector T cells, although this is still controversial. Tregs can also present defective activation. In peripheral lymphoid organs, the transition of T cells to PILs is under control of miRNAs that can modulate the expression of chemokine receptors and consequent migration of these cells to the pancreas. Together, these changes may possibly be due to intrathymic hormonal imbalance, comprising the expression of insulin, IGF-1 and IGF-2.

The role of IGF-2 specifically in the thymus of NOD mice is not yet defined. However, transcriptome studies revealed that IGF-2 mRNA is downregulated when comparing mTECs from newborn and 5 week old NOD mice (75). A downmodulation was also observed for Ins1 and Ins2 mRNA expression. Interestingly, the same was observed in BALB/c mice for both IGF-2 and Ins-2, but not for Ins-1 mRNA. Moreover, the reconstruction of post-transcriptional miRNA-mRNA interaction networks revealed that some miRNAs, including the miR-647 that targets IGF-2 mRNA, were included in the network of BALB/c, but not in the NOD mice (75). In this context, since the expression of PTA mRNAs (and the respective proteins) in mTECs is important for the negative selection process, the mechanisms that inhibit the regulatory action of miRNAs may be acting in these cells.

The expression of other miRNAs is altered in the thymus of NOD mice when comparing with C57BL/6 mice (**Table 1**). The miR-19a, miR-19b and miR-326 are downregulated whereas miR-124a is upregulated on TCR^{+} thymocytes. MiR-133b is upregulated only in natural killer T (NKT) cells in the thymus (67). This miRNA targets and regulates the transcription factor Th-POK, which negatively regulates the differentiation of IL-17 producing NKT cells (NKT17). Thus, the diminished expression of Th-POK can induce the differentiation of NKT17 cells and explain the enhanced numbers of these cells in the thymus and peripheral lymphoid organs of NOD mice (67), which can be related with exacerbation of diabetes (76).

THE ROLE OF MIRNAS THROUGHOUT TRANSITION OF THYMOCYTES INTO PANCREAS INFILTRATING LYMPHOCYTES

During the period of evolution of autoimmune reactivity in NOD mice, even before the appearance of clinical signs of T1D, thymocytes that differentiate into peripheral $CD3^+$ T lymphocytes sequentially modulate (up- or down-regulate), a significant set of mRNAs that encode proteins involved in the intrathymic negative selection, T cell maturation, differentiation and autoreactivity (77). Among the peripheral T lymphocytes residing in the spleen and/or in the lymph nodes some autoreactive clones will evolve into PILs in mice and humans (1, 78, 79). Insulin-specific CD4 and CD8 T cells targeting multiple epitopes are predominant in the islet infiltrating T cells in pre-diabetic NOD mice pancreas (80), with proinsulin 2 being proposed as the major isoform recognized by those cells (33).

During the transition of peripheral T lymphocytes into PILs, a large set of mRNAs is transcriptionally modulated causing changes in the transcriptome profile of these cells with parallel modulation of miRNAs. These transcriptional changes are robust enough to hierarchize the different cell types (thymocytes, $CD3^+$ peripheral T lymphocytes and PILs) and the different stages of NOD mice regarding the onset of T1D (pre- or diabetic animals) according to their respective mRNA or miRNA expression signatures (81). The miRNA modulation strongly suggests that transition into PILs would be under posttranscriptional control, i.e., the effect of specific miRNAs upon target mRNAs that encode proteins involved in this process.

The reconstruction of miRNA-mRNA interaction networks, based on differential expression profiling of peripheral T lymphocytes during their transition into PILs in NOD mice predicted mRNA targets in an unbiased way. As these cells develop into $CD3^+$ peripheral T cells and then into PILs, thymocytes exhibited miRNA interactions with mRNA targets that encode proteins related to apoptosis, cell adhesion, positive and negative selection in the thymus. The interactions involving miR-202-3p with CCR7 mRNA were highlighted in the work of Fornari et al. (81), showing that CCR7 is involved with the control of central tolerance and mice lacking this chemokine receptor generated autoreactive T cells (82). Moreover, CCR7 directs T-cells toward the pancreas of NOD mice, since desensitization of CCR7 blocked T-cell migration from the bloodstream into pancreatic islets (83).

A second interaction emphasized was miR-202-3p-CD247 mRNA in NOD mice (81). Under disturbance during TCR signaling, the CD3 zeta chain enhanced autoimmune diabetes in mice (84).

The evidence at this moment suggests that the transition into PILs is under post-transcriptional control exerted by miRNAs (**Figure 1**). Interestingly, some miRNAs such as miR-375, miR-30d and miR-9, can control insulin synthesis and secretion by pancreatic beta-cells (85) in NOD mice. Whether miRNAs also regulate the intrathymic production of proinsulin/insulin and IGF remains unknown.

FUTURE DEVELOPMENTS AND CONCLUDING REMARKS

Although the precise biological mechanism(s) underlying how differentiating thymocytes evolve to autoreactive T-cells infiltrating and destroying pancreatic beta cells are not elucidated, it is likely that disturbances of gene and miRNA signatures may be part of this process, as well as changes in the profiles of cell migration of both thymocytes and peripheral T lymphocytes. In this context, besides the questions raised throughout the text, other important questions remain unanswered, such as the possible direct role of the thymic alterations in the pathogenesis of T1D and the presence of similar alterations in humans.

In humans, serum GH levels are enhanced in T1D patients (86), and IGF-1 and IGF-1R mRNA levels are reduced in peripheral blood mononuclear cells (87). The circulation levels of GH are enhanced whereas IGF-1 levels are diminished in NOD diabetic mice 4 weeks after the appearance of glycosuria (88), suggesting similarities in hormone imbalance between T1D patients and NOD mice at least after disease diagnosis.

Hormonal imbalance in the thymus can be involved in the control of the physiology of the organ in NOD mice and humans, as the properly maturation of the cells, cell adhesion, migration, accumulation and egress, by the modulation of ECM molecules and integrins, chemokines and chemokine receptors, sphingosine-1-phosphate and sphingosine-1-phosphate receptor 1 (10, 64, 65, 89). As an example, GH/IGF-1 axis can modulate the expression of cytokines, chemokines and ECM molecules and receptors in the thymus (61). GH modulates thymocyte adhesion and migration properties, and promotes thymocyte egress (59). The effects of GH can be regulated by IGF-1, which can in turn bind IGF-R and insulin receptor (90). Lower insulin levels in the thymus are related with reactivity to insulin in the periphery, including in NOD mice (33). Together, these mechanisms can shape the T cell repertoire and change the frequency of Tregs and the ratio of Treg and effector T cells (34, 45). The diminished frequency of Tregs in NOD mice is controversial, and most studies in T1D patients have reported no differences in the frequency of Tregs in peripheral blood. Likewise, phenotype and diminished suppressive capacity have been reported in both NOD and T1D patients (64, 91–93). Whether these specific issues are related with hormonal imbalance during the pathogenesis of T1D, comprising the expression of insulin, GH/IGF-1 and IGF-2, need further investigation.

AUTHOR'S NOTE

This manuscript is dedicated to Prof. Mireille Dardenne, for her significant contribution in the field of Immunoendocrinology along the last 50 years. In 2017 we celebrated her 80th birthday.

AUTHOR CONTRIBUTIONS

All authors listed conceived and wrote the manuscript, and approved it for publication.

FUNDING

This work was developed in the context of the National Institute of Science and Technology on Neuroimmunomodulation (INCT-NIM) and was partially funded with grants from the Oswaldo Cruz Foundation, the Brazilian National Council for Development of Science and Technology (CNPq) grant # 306315/2013-0 to GP, Coordination for the Improvement of Higher Education Personnel (CAPES) grant # 88881.068105/2014-01 to GP, State of Rio de Janeiro Foundation for Funding Research (FAPERJ), State of São Paulo Research Foundation (FAPESP) grant # 13/17481-1 to GP, and the Mercosur Program for Structural Convergence (FOCEM #03/11).

REFERENCES

1. Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. *Lancet* (2014) 383:69–82. doi: 10.1016/S0140-6736(13)60591-7
2. Lampasona V, Liberati D. Islet autoantibodies. *Curr Diab Rep.* (2016) 16:53. doi: 10.1007/s11892-016-0738-2
3. Ziegler A, Hummel M, Schenker M, Bonifacio E. Autoantibody appearance and risk for development of childhood diabetes in offspring of parents with type 1 diabetes the 2-year analysis of the German BABYDIAB study. *Diabetes* (1999) 48:460–8.
4. Cheng B-W, Lo F-S, Wang A-M, Hung C-M, Huang C-Y, Ting W-H, et al. Autoantibodies against islet cell antigens in children with type 1 diabetes mellitus. *Oncotarget* (2018) 9:16275–83. doi: 10.18632/oncotarget.24527
5. Willcox A, Richardson SJ, Bone AJ, Foulis AK, Morgan NG. Analysis of islet inflammation in human type 1 diabetes. *Clin Exp Immunol.* (2009) 155:173–81. doi: 10.1111/j.1365-2249.2008.03860.x
6. Bottazzo GF, Dean BM, McNally JM, MacKay EH, Swift PG, Gamble DR. *In situ* characterization of autoimmune phenomena and expression of HLA molecules in the pancreas in diabetic insulitis. *N Engl J Med.* (1985) 313:353–60. doi: 10.1056/NEJM198508083130604
7. Pearson JA, Wong FS, Wen L. The importance of the Non Obese Diabetic (NOD) mouse model in autoimmune diabetes. *J Autoimmun.* (2016) 66:76–88. doi: 10.1016/j.jaut.2015.08.019
8. Burrack AL, Martinov T, Fife BT. T cell-mediated beta cell destruction: autoimmunity and alloimmunity in the context of type 1 diabetes. *Front Endocrinol.* (2017) 8:343. doi: 10.3389/fendo.2017.00343
9. Boursalian TE, Golob J, Soper DM, Cooper CJ, Fink PJ. Continued maturation of thymic emigrants in the periphery. *Nat Immunol.* (2004) 5:418–25. doi: 10.1038/ni1049
10. Savino W, Mendes-da-Cruz DA, Lepletier A, Dardenne M. Hormonal control of T-cell development in health and disease. *Nat Rev Endocrinol.* (2016) 12:77–89. doi: 10.1038/nrendo.2015.168
11. Ohkura N, Sakaguchi S. Regulatory T cells: roles of T cell receptor for their development and function. *Semin Immunopathol.* (2010) 32:95–106. doi: 10.1007/s00281-010-0200-5
12. Josefowicz SZ, Lu L-F, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. *Annu Rev Immunol.* (2012) 30:531–64. doi: 10.1146/annurev.immunol.25.022106.141623
13. Richards DM, Delacher M, Goldfarb Y, Kägebein D, Hofer A-C, Abramson J, et al. Treg cell differentiation: from thymus to peripheral tissue. *Prog Mol Biol Trans Sci.* (2015) 136:175–205. doi: 10.1016/bs.pmbts.2015.07.014
14. Geenen V. Thymus and type 1 diabetes: an update. *Diabetes Res Clin Pract.* (2012) 98:26–32. doi: 10.1016/j.diabres.2012.05.023
15. Anderson MS, Venanzi ES, Klein L, Chen Z, Berzins SP, Turley SJ, et al. Projection of an immunological self shadow within the thymus by the aire protein. *Science* (2002) 298:1395–401. doi: 10.1126/science.1075958
16. Passos GA, Mendes-da-Cruz DA, Oliveira EH. The thymic orchestration involving Aire, miRNAs and cell-cell interactions during the induction of central tolerance. *Front Immunol.* (2015) 6:352. doi: 10.3389/fimmu.2015.00352
17. Ahonen P. Autoimmune polyendocrinopathy - candidosis - ectodermal dystrophy (APECED): autosomal recessive inheritance. *Clin Genet.* (1985) 27:429–32. doi: 10.1016/B978-1-4557-4975-1.00061-9
18. Nagamine K, Peterson P, Scott HS, Kudoh J, Minoshima S, Heino M, et al. Positional cloning of the APECED gene. *Nat Genet.* (1997) 17:393–8. doi: 10.1038/ng1297-393
19. Levi D, Polychronakos C. Expression profile of a clonal insulin-expressing epithelial cell in the thymus. *Mol Immunol.* (2013) 56:804–10. doi: 10.1016/j.molimm.2013.07.015.Expression
20. Ramsey C, Winqvist O, Puhakka L, Halonen M, Moro A, Kämpe O, et al. Aire deficient mice develop multiple features of APECED phenotype and show altered immune response. *Hum Mol Genet.* (2002) 11:397–409. doi: 10.1093/hmg/11.4.397
21. Pugliese A. The insulin gene in type 1 diabetes. *IUBMB Life* (2005) 57:463–8. doi: 10.1080/15216540500163301
22. Kecha-Kamoun O, Achour I, Martens H. Thymic expression of insulin-related genes in an animal model of autoimmune type 1 diabetes. *Diabetes Metab Res Rev.* (2001) 17:146–52. doi: 10.1002/dmrr.182
23. Kecha O, Brilot F, Martens H, Franchimont N, Renard C, Greimers R, et al. Involvement of insulin-like growth factors in early T cell development: a study using fetal thymic organ cultures. *Endocrinology* (2000) 141:1209–17. doi: 10.1210/endo.141.3.7360
24. Carlsén M, Cilio CM. Normal T cell development in the absence of thymic insulin expression. *Ann NY Acad Sci.* (2006) 1079:205–12. doi: 10.1196/annals.1375.032
25. Vafiadis P, Bennett ST, Todd JA, Nadeau J, Grabs R, Goodyer CG, et al. Insulin expression in human thymus is modulated by INS VNTR alleles at the IDDM2 locus. *Nat Genet.* (1997) 15:289–92. doi: 10.1038/ng0397-289
26. Bennett ST, Todd JA. Human type 1 diabetes and the insulin gene: principles of mapping polygenes. *Annu Rev Genet.* (1996) 30:343–70.
27. Pugliese A, Zeller M, Fernadez A Jr, Zalcberg LJ, Bartlett RJ, Ricordi C, et al. The insulin gene is transcribed in the human thymus and transcription levels correlate with allelic variation at the INS VNTR-IDDM2 susceptibility locus for type 1 diabetes. *Nat Genet.* (1997) 15:293–7. doi: 10.1038/ng0797-270
28. Wentworth BM, Schaefer IM, Villa-Komaroff L, Chirgwin JM. Characterization of the two nonallelic genes encoding mouse preproinsulin. *J Mol Evol.* (1986) 23:305–12. doi: 10.1007/BF02100639
29. Duvillié B, Cordonnier N, Deltour L, Dandoy-Dron F, Itier JM, Monthioux E, et al. Phenotypic alterations in insulin-deficient mutant mice. *Proc Natl Acad Sci USA.* (1997) 94:5137–40. doi: 10.1073/pnas.94.10.5137
30. Derbinski J, Schulte A, Kyewski B, Klein L. Promiscuous gene expression in medullary thymic epithelial cells mirrors the peripheral self. *Nat Immunol.* (2001) 2:2915–22. doi: 10.1038/ni723
31. Chentoufi AA, Palumbo M, Polychronakos C. Proinsulin expression by Hassall's corpuscles in the mouse thymus. *Diabetes* (2004) 53:354–9. doi: 10.2337/diabetes.53.2.354
32. Kermani H, Goffinet L, Mottet M, Bodart G, Morrhaye G, Dardenne O, et al. Expression of the growth hormone/insulin-like growth factor axis during Balb/c thymus ontogeny and effects of growth hormone upon *ex vivo* T cell differentiation. *Neuroimmunomodulation* (2012) 19:137–47. doi: 10.1159/000328844
33. Thébault-baumont K, Dubois-laforgue D, Krief P, Briand J, Halbout P, Vallon-geoffroy K, et al. Acceleration of type 1 diabetes mellitus in proinsulin 2 – deficient NOD mice. *J Clin Invest.* (2003) 111:851–7. doi: 10.1172/JCI200316584.Introduction
34. Chentoufi AA, Polychronakos C. Insulin expression levels in the thymus modulate insulin-specific autoreactive t-cell tolerance the mechanism by which the IDDM2 locus may predispose to diabetes. *Diabetes* (2002) 51:1383–90. doi: 10.2337/diabetes.51.5.1383
35. Faideau B, Lotton C, Lucas B, Tardivel I, Elliott JF, Boitard C, et al. Tolerance to proinsulin-2 is due to radioresistant thymic cells. *J Immunol.* (2006) 177:53–60. doi: 10.4049/jimmunol.177.1.53

36. Baker J, Liu JP, Robertson EJ, Efstratiadis A. Role of insulin-like growth factors in embryonic and postnatal growth. *Cell* (1993) 75:73–82. doi: 10.1016/0092-8674(93)90680-O
37. Neely EK, Beukers MW, Oh Y, Cohen P, Rosenfeld RG. Insulin-like growth factor receptors. *Acta Paediatr Scand.* (1991) 372:116–23.
38. Geenen V, Achour I, Robert F, Vandersmissen E, Sodoyez J-C, Defresne M-P, et al. Evidence that insulin-like growth factor 2 (IGF2) is the dominant thymic peptide of the insulin superfamily. *Thymus* (1993) 21:115–27.
39. Geenen V, Kecha O, Brilot F, Charlet-Renard C, Martens H. The thymic repertoire of neuroendocrine-related self antigens: biological role in T-cell selection and pharmacological implications. *Neuroimmunomodulation* (1999) 6:115–25. doi: 10.1159/000026371
40. Jolicoeur C, Hanahan D, Smith KM. T-cell tolerance toward a transgenic beta-cell antigen and transcription of endogenous pancreatic genes in thymus. *Proc Natl Acad Sci USA.* (1994) 91:6707–11. doi: 10.1073/pnas.91.14.6707
41. Geenen V. The thymic insulin-like growth factor axis : involvement in physiology and disease. *Horm Metab Res.* (2003) 35:1–8. doi: 10.1055/s-2004-814161
42. Geenen V, Lefebvre PJ. The intrathymic expression of insulin-related genes: implications for pathophysiology and prevention of Type 1 diabetes. *Diabetes Metab Rev.* (1998) 14:95–103.
43. Savino W, Cotta-de-Almeida V, Van Buul-Offers SC, Koster JG, Dardenne M. Abnormal thymic microenvironment in insulin-like growth factor-II transgenic mice. *Neuroimmunomodulation* (2005) 12:100–12. doi: 10.1159/000083582
44. Brissenden JE, Ullrich A, Francke U. Human chromosomal mapping of genes for insulin-like growth factors I and II and epidermal growth factor. *Nature* (1984) 310:781–4. doi: 10.1038/310781a0
45. Chentoufi AA, Geenen V. Thymic self-antigen expression for the design of a negative/tolerogenic self-vaccine against type 1 diabetes. *Clin Dev Immunol.* (2011) 2011: 349368. doi: 10.1155/2011/349368
46. Zapf J, Walter H, Froesch ER. Radioimmunological determination of insulinlike growth factors I and II in normal subjects and in patients with growth disorders and extrapancreatic tumor hypoglycemia. *J Clin Invest.* (1981) 68:1321–30. doi: 10.1172/JCI110379
47. Shimatsu A, Nakamura Y, Ihara C, Mizuta H, Murabe H. Serum Insulin-like growth factor-II (IGF-II) levels in patients with acromegaly and growth hormone deficiency: correlation with IGF-I and IGF binding protein-3. *Endocr J.* (1996) 43:S71-3.
48. Hansenne I, Renard-Charlet C, Greimers R, Geenen V. Dendritic cell differentiation and immune tolerance to insulin-related peptides in Igf2-deficient mice. *J Imm.* (2006) 176:4651–7. doi: 10.4049/jimmunol.176.8.4651
49. Timsit J, Savino W, Safieh B, Chanson P, Gagnerault M-C, Bach J-F, et al. Growth hormone and insuli-like growth factor-I stimulate hormonal function and proliferation of thymic epithelial cells *. *J Clin Endocrinol Metab.* (1992) 75:183–8. doi: 10.1210/jcem.75.1.1619008
50. Welniak LA, Sun R, Murphy WJ. The role of growth hormone in T-cell development and reconstitution. *J Leukoc Biol.* (2002) 71:381–7. doi: 10.1189/jlb.71.3.381. (Accessed January 30, 2018).
51. de Mello-Coelho V, Gagnerault M-C, Souberbielle J-C, Strasburger CJ, Savino W, Dardenne M, et al. Growth hormone and its receptor are expressed in human thymic cells. *Endocrinology* (1998) 139:3837–42. doi: 10.1210/endo.139.9.6199
52. Pierpaoli W, Sorkin E. Hormones and immunologic capacity. I. effect of heterologous anti-growth hormone (ASTH) antiserum on thymus and peripheral lymphatic tissue in mice. Induction of a wasting syndrome. *J Immunol.* (1968) 101:1036–43. (Accessed January 30, 2018).
53. Mertani HC, Delehaye-Zervas MC, Martini JF, Postel-Vinay MC, Morel G. Localization of growth hormone receptor messenger RNA in human tissues. *Endocrine* (1995) 3:135–42. doi: 10.1007/BF02990065
54. Mertani HC, Morel G. *In situ* gene expression of growth hormone (GH) receptor and GH binding protein in adult male rat tissues. *Mol Cell Endocrinol.* (1995) 109:47–61. (Accessed January 30, 2018).
55. Gagnerault MC, Postel-Vinay MC, Dardenne M. Expression of growth hormone receptors in murine lymphoid cells analyzed by flow cytofluorometry. *Endocrinology* (1996) 137:1719–26. doi: 10.1210/endo.137.5.8612507
56. Hirokawa K, Utsuyama M, Kikuchi Y. Trade off situation between thymus and growth hormone: age-related decline of growth hormone is a cause of thymic involution but favorable for elongation of lifespan. *Biogerontology* (2016) 17:55–9. doi: 10.1007/s10522-015-9590-z
57. Savino W, Smaniotto S, Mendes-da-Cruz DA, Dardenne M. Growth hormone modulates migration of thymocytes and peripheral T cells. *Ann NY Acad Sci.* (2012) 1261:49–54. doi: 10.1111/j.1749-6632.2012.06637.x
58. Smaniotto S, Ribeiro-Carvalho MM, Dardenne M, Savino W, de Mello-Coelho V. Growth hormone stimulates the selective trafficking of thymic CD4+CD8- emigrants to peripheral lymphoid organs. *Neuroimmunomodulation* (2004) 11:299–306. doi: 10.1159/000079410
59. Smaniotto S, De Mello-Coelho V, Villa-Verde DMS, Pléau JM, Postel-Vinay MC, Dardenne M, et al. Growth hormone modulates thymocyte development *in vivo* through a combined action of laminin and CXC chemokine ligand 12. *Endocrinology* (2005) 146:3005–17. doi: 10.1210/en.2004-0709
60. Lins MP, de Araújo Vieira LF, Rosa AAM, Smaniotto S. Growth hormone in the presence of laminin modulates interaction of human thymic epithelial cells and thymocytes *in vitro*. *Biol Res.* (2016) 49:37. doi: 10.1186/s40659-016-0097-0
61. de Mello-Coelho V, Villa-Verde DM, Dardenne M, Savino W. Pituitary hormones modulate cell-cell interactions between thymocytes and thymic epithelial cells. *J Neuroimmunol.* (1997) 76:39–49. (Accessed August 5, 2015).
62. Savino W, Boitard C, Bach JF, Dardenne M. Studies on the thymus in nonobese diabetic mouse. I. Changes in the microenvironmental compartments. *Lab Invest.* (1991) 64:405–17. (Accessed August 5, 2015).
63. Savino W, Carnaud C, Luan JJ, Bach JF, Dardenne M. Characterization of the extracellular matrix-containing giant perivascular spaces in the NOD mouse thymus. *Diabetes* (1993) 42:134–40. (Accessed August 5, 2015).
64. Mendes-da-Cruz DA, Smaniotto S, Keller AC, Dardenne M, Savino W. Multivectorial abnormal cell migration in the NOD mouse thymus. *J Immunol.* (2008) 180:4639–47. doi: 10.4049/jimmunol.180.7.4639. (Accessed August 10, 2016).
65. Cotta-de-Almeida V, Villa-Verde DMS, Lepault F, Pléau J-M, Dardenne M, Savino W. Impaired migration of NOD mouse thymocytes: a fibronectin receptor-related defect. *Eur J Immunol.* (2004) 34:1578–87. doi: 10.1002/eji.200324765
66. Brimnes MK, Jensen T, Jørgensen TN, Michelsen BK, Troelsen J, Werdelin O. Low expression of insulin in the thymus of non-obese diabetic mice. *J Autoimmun.* (2002) 19:203–13. doi: 10.1006/jaut.2002.0616
67. Di Pietro C, De Giorgi L, Cosorich I, Sorini C, Fedeli M, Falcone M. MicroRNA-133b regulation of Th-POK expression and dendritic cell signals affect NKT17 cell differentiation in the thymus. *J Immunol.* (2016) 197:3271–80. doi: 10.4049/jimmunol.1502238
68. Chen W, Bergerot I, Elliott JF, Harrison LC, Abiru N, Eisenbarth GS, et al. Evidence that a peptide spanning the B-C junction of proinsulin is an early Autoantigen epitope in the pathogenesis of type 1 diabetes. *J Immunol.* (2001) 167:4926–35. doi: 10.4049/jimmunol.167.9.4926
69. Moriyama H, Abiru N, Paronen J, Sikora K, Liu E, Miao D, et al. Evidence for a primary islet autoantigen (preproinsulin 1) for insulitis and diabetes in the nonobese diabetic mouse. *Proc Natl Acad Sci USA.* (2003) 100:10376–81. doi: 10.1073/pnas.1834450100
70. French MB, Allison J, Cram DS, Thomas HE, Dempsey-collier M, Silva A, et al. Ttaisgenic expression of mouse proinsulin II prevents diabetes in nonobese diabetic mice. *Diabetes* (1997) 46:34–9.
71. Cetkovic-Cvrlje M, Gerling IC, Muir A, Atkinson MA, Elliott JF, Leiter EH. Retardation or acceleration of diabetes in NOD/Lt mice mediated by intrathymic administration of candidate β-cell antigens. *Diabetes* (1997) 46:1975–82.
72. Pearson JA, Thayer TC, McLaren JE, Ladell K, De Leenheer E, Phillips A, et al. Proinsulin expression shapes the TCR repertoire but fails to control the development of low-avidity insulin-reactive CD8 $^{+}$ T cells. *Diabetes* (2016) 65:1679–89. doi: 10.2337/db15-1498
73. Bergerot I, Fabien N, Maguer V, Thivolet C. Insulin-like growth factor-1 (IGF-1) protects NOD mice from insulitis and diabetes. *Clin Exp Immunol.* (1995) 102:335–40. (Accessed January 30, 2018).

74. Bernacchioni C, Cencetti F, Blescia S, Donati C, Bruni P. Sphingosine kinase/sphingosine 1-phosphate axis: a new player for insulin-like growth factor-1-induced myoblast differentiation. *Skelet Muscle* (2012) 2:15. doi: 10.1186/2044-5040-2-15
75. Macedo C, Oliveira EH, Almeida RS, Donate PB, Fornari TA, Pezzi N, et al. Aire-dependent peripheral tissue antigen mRNAs in mTEC cells feature networking refractoriness to microRNA interaction. *Immunobiology* (2015) 220:93–102. doi: 10.1016/j.imbio.2014.08.015
76. Simoni Y, Gautron A-S, Beaudoin L, Bui L-C, Michel M-L, Coumoul X, et al. NOD mice contain an elevated frequency of iNKT17 cells that exacerbate diabetes. *Eur J Immunol.* (2011) 41:3574–85. doi: 10.1002/eji.201141751
77. Fornari TA, Donate PB, Macedo C, Sakamoto-Hojo ET, Donadi EA, Passos GA. Development of type 1 diabetes mellitus in nonobese diabetic mice follows changes in thymocyte and peripheral T lymphocyte transcriptional activity. *Clin Dev Immunol.* (2011) 2011:1–12. doi: 10.1155/2011/158735
78. Michels AW, Landry LG, McDaniel KA, Yu L, Campbell-Thompson M, Kwok WW, et al. Islet-derived CD4 T cells targeting proinsulin in human autoimmune diabetes. *Diabetes* (2017) 66:722–34. doi: 10.2337/db16-1025
79. Serreze D V, Leiter EH. Genetic and pathogenic basis of autoimmune diabetes in NOD mice. *Curr Opin Immunol.* (1994) 6:900–6. (Accessed January 29, 2018).
80. Wegmann DR, Gill RG, Norbury-Glaser M, Schloot N, Daniel D. Analysis of the spontaneous T cell response to insulin in NOD mice. *J Autoimmun.* (1994) 7:833–43.
81. Fornari TA, Donate PB, Assis AF, Macedo C, Sakamoto-Hojo ET, Donadi EA, et al. Comprehensive survey of miRNA-mRNA interactions reveals that Ccr7 and Cd247 (CD3 zeta) are posttranscriptionally controlled in pancreas infiltrating T lymphocytes of Non-Obese Diabetic (NOD) mice. *PLoS ONE* (2015) 10:e0142688. doi: 10.1371/journal.pone.0142688
82. Martin AP, Marinkovic T, Canasto-Chibuque C, Latif R, Unkeless JC, Davies TF, et al. CCR7 deficiency in NOD mice leads to thyroiditis and primary hypothyroidism. *J Immunol.* (2009) 183:3073–80. doi: 10.4049/jimmunol.0900275
83. Shan Z, Xu B, Mikulowska-Mennis A, Michie SA. CCR7 directs the recruitment of T cells into inflamed pancreatic islets of nonobese diabetic (NOD) mice. *Immunol Res.* (2014) 58:351–7. doi: 10.1007/s12026-014-8500-9
84. Lundholm M, Mayans S, Motta V, Löfgren-Burström A, Danska J, Holmberg D. Variation in the Cd3 zeta (Cd247) gene correlates with altered T cell activation and is associated with autoimmune diabetes. *J Immunol.* (2010) 184:5537–44. doi: 10.4049/jimmunol.0904012
85. Zheng Y, Wang Z, Zhou Z. miRNAs: novel regulators of autoimmunity-mediated pancreatic β-cell destruction in type 1 diabetes. *Cell Mol Immunol.* (2017) 14:488–96. doi: 10.1038/cmi.2017.7
86. Hayford JT, Danney MM, Hendrix JA, Thompson RG. Integrated concentration of growth hormone in juvenile-onset diabetes. *Diabetes* (1980) 29:391–8. (Accessed May 21, 2018).
87. de Souza KSC, Ururahy MAG, da Costa Oliveira YM, Loureiro MB, da Silva HPV, Bortolin RH, et al. Low bone mineral density in patients with type 1 diabetes: association with reduced expression of *IGF1* , *IGF1R* and *TGF B 1* in peripheral blood mononuclear cells. *Diabetes Metab Res Rev.* (2016) 32:589–95. doi: 10.1002/dmrr.2772
88. Landau D, Segev Y, Eshet R, Flyvbjerg A, Phillip M. Changes in the growth hormone-IGF-I axis in non-obese diabetic mice. *Int J Exp Diabetes Res.* (2000) 1:9–18. doi: 10.1155/EDR.2000.9 (Accessed May 20, 2018).
89. Lemos J, Smaniotto S, Messias C, Moreira O, Cotta-de-Almeida V, Dardenne M, et al. Sphingosine-1-phosphate receptor 1 is involved in non-obese diabetic mouse thymocyte migration disorders. *Int J Mol Sci.* (2018) 19:1446. doi: 10.3390/ijms19051446
90. de Mello Coelho V, Villa-Verde DMS, Farias-de-Oliveira DA, Marques de Brito J, Dardenne M, Savino W. Functional insulin-like growth factor-1/insulin-like growth factor-1 receptor-mediated circuit in human and murine thymic epithelial cells. *Clin Neuroendocrinol.* (2002) 75:139–50. doi: 10.1159/000048230
91. Ferreira C, Palmer D, Blake K, Garden OA, Dyson J. Reduced regulatory T cell diversity in NOD mice is linked to early events in the thymus. *J Immunol.* (2014) 192:4145–52. doi: 10.4049/jimmunol.1301600
92. Hull CM, Peakman M, Tree TIM. Regulatory T cell dysfunction in type 1 diabetes: what's broken and how can we fix it? *Diabetologia* (2017) 60:1839–50. doi: 10.1007/s00125-017-4377-1
93. Visperas A, Vignali DAA. Are regulatory T cells defective in type 1 diabetes and can we fix them? *J Immunol.* (2016) 197:3762–70. doi: 10.4049/jimmunol.1601118

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling Editor is currently co-organizing a Research Topic with the authors DMC, WS and confirms the absence of any other collaboration.

frontiers in Endocrinology

REVIEW
published: 17 May 2018
doi: 10.3389/fendo.2018.00229

Interactions Between the Neuroendocrine System and T Lymphocytes in Diabetes

*Luz Andreone, María Laura Gimeno and Marcelo J. Perone**

Instituto de Investigación en Biomedicina de Buenos Aires (IBioBA) – CONICET – Instituto Partner de la Sociedad Max Planck, Buenos Aires, Argentina

It is well established that there is a fine-tuned bidirectional communication between the immune and neuroendocrine tissues in maintaining homeostasis. Several types of immune cells, hormones, and neurotransmitters of different chemical nature are involved as communicators between organs. Apart of being key players of the adaptive arm of the immune system, it has been recently described that T lymphocytes are involved in the modulation of metabolism of several tissues in health and disease. Diabetes may result mainly from lack of insulin production (type 1 diabetes) or insufficient insulin and insulin resistance (type 2 diabetes), both influenced by genetic and environmental components. Herein, we discuss accumulating data regarding the role of the adaptive arm of the immune system in the pathogenesis of diabetes; including the action of several hormones and neurotransmitters influencing on central and peripheral T lymphocytes development and maturation, particularly under the metabolic burden triggered by diabetes. In addition, we comment on the role of T-effector lymphocytes in adipose and liver tissues during diabetes, which together enhances pancreatic β-cell stress aggravating the disease.

Keywords: T lymphocytes, inflammation, insulin resistance, adipose, muscle, liver, cytokines

OPEN ACCESS

Edited by:
Daniella Areas Mendes-Da-Cruz, Fundação Oswaldo Cruz (Fiocruz), Brazil

Reviewed by:
Denis Richard, Laval University, Canada
Licio A. Velloso, Universidade Estadual de Campinas, Brazil

***Correspondence:**
Marcelo J. Perone
peronemj@gmail.com

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: *30 December 2017*
Accepted: *20 April 2018*
Published: *17 May 2018*

Citation:
Andreone L, Gimeno ML and Perone MJ (2018) Interactions Between the Neuroendocrine System and T Lymphocytes in Diabetes. Front. Endocrinol. 9:229. doi: 10.3389/fendo.2018.00229

INTRODUCTION

Pioneering work in the 1980s provided the first evidence of the cross-talk between the neuroendocrine and immune systems (1–4). It is now well established that there is a fine-tuned bidirectional communication between these tissues in maintaining homeostasis. Several types of immune cells, hormones, and neurotransmitters of different chemical nature are involved as communicators between organs influencing immune development and function (5, 6). Additionally, it has been described that T lymphocytes apart of being key players of the adaptive arm of the immune system, are involved in the modulation of metabolism in several tissues in health and disease (7–13).

Diabetes is a highly prevalent endocrine-metabolic disease with a constant growing rate, affecting nearly half a billion people worldwide (14). It is characterized by an imbalance in glucose homeostasis, which result mainly from lack of insulin production in the pancreas [type 1 diabetes (T1D)] or insufficient insulin production and peripheral insulin resistance [type 2 diabetes (T2D)] both influenced by genetic and environmental components.

In this Review, we discuss existing data about the role of the adaptive arm of the immune system in the diabetes pathophysiology; including the action of several hormones and neurotransmitters influencing on central and peripheral T lymphocytes development and maturation, particularly under the metabolic burden triggered by diabetes. In addition, we comment on the role of T-effector

lymphocytes in peripheral tissues during diabetes, which together enhance pancreatic β-cell stress aggravating the disease.

THE ROLE OF T CELLS IN THE PATHOGENESIS OF T1D

Type 1 diabetes is a T cell-mediated autoimmune disease that selectively destroys insulin-producing β-cells. The key roles for both $CD4^+$ and $CD8^+$ T cells in the immune response that drives T1D have been extensively described (15, 16). It is now widely accepted that endogenous and/or exogenous initiating factors, operating on a genetic susceptibility background and permissive environmental framework, are necessary for the development of autoreactive T lymphocytes that infiltrate pancreatic islets (insulitis) (17).

While the association of class II HLA genes polymorphisms with T1D risk has been known for over 40 years (18), recent single-nucleotide polymorphisms (SNPs) genotyping technologies allow the description of many additional T1D susceptibility genes (19–21). Intriguingly, most of these genes are coding for cytokines, cytokine receptors, and factors that regulate T cell differentiation, suggesting that control of T cell identity may be an important element of the genetic contribution to disease susceptibility and onset.

The process of T cell differentiation that takes place in the thymus is regulated by many molecules such as hormones, neuropeptides, and neurotransmitters involving both endocrine and paracrine signaling pathways (6). A variety of peptide and nonpeptide hormones modulate the proliferation, differentiation, migration, and apoptosis of developing thymocytes. The dysfunction in the hormonal control of T cell differentiation is associated with the development of diseases that are influenced by immune cells, including diabetes.

Currently, there is a wide consensus that T1D is a Th1-mediated pathology and INF-γ is implicated as the main driver cytokine of the process of autoimmune islet destruction; meanwhile, Th2 cell-type would play a protective role (22–29). However, not all emerging data from mouse models and patients are consistent with the dominance of a Th1 response in T1D; multiple additional T cell differentiation phenotypes are now recognized with distinct functions (30, 31).

The role of Th17 lymphocytes in T1D is not fully understood. Murine models and human studies suggest that IL-17 is upregulated in the early stages of diabetes development but it is still not clear if this cytokine, or indeed if the Th17 subset, is necessary for disease (32–38). It was shown that genetic IL-17 silencing had no effect and did not protect NOD mice from spontaneous autoimmune diabetes (39). Some studies suggested that an increase of T cells co-expressing IFN-γ and IL-17 could be a feature of T1D development (36, 40–42). Several types of cells of the immune system, attracted by signals from the islets, contribute to the selective β-cell death through the release of cytotoxic inflammatory cytokines, such as IL-1β, IFN-γ, and TNF-α (43, 44). Recent studies performed in human β-cells suggested that pancreatic IL-17 contributes to the pathogenesis of T1D by two mechanisms, exacerbating β-cell apoptosis and increasing local production of chemokines by islets exposed to pro-inflammatory cytokines (e.g., IL-1β + IFN-γ and TNF-α + IFN-γ) (45). In a study of children in various phases of diabetes-associated autoimmunity and clinical disease upregulation of IL-17 and Th1/Th17 plasticity in peripheral blood were observed in stages of advanced β-cell autoimmunity and impaired glucose tolerance and clinical T1D (42). Activated Th17 immunity was not observed in patients with early β-cell autoimmunity, indicating that Th17 may be a marker of late preclinical autoimmune diabetes which correlates with impaired β-cell function. Analysis of pancreatic lymph nodes in T1D patients showed higher frequency of Th17 cells in comparison with non-diabetic controls (46). The consolidation of Th17 cells as part of T1D pathophysiology focused attention on additional cytokines, outside of those associated classically with the Th1/Th2 paradigm (IFN-γ and IL-4, respectively).

IL-21 is a pleiotropic cytokine produced mainly by T follicular helper (Tfh) cells, Th17 cells, and natural killer (NK) cells. Although it has been demonstrated that IL-21 enhances Th17 differentiation and it can be produced by Th17 cells to exert autocrine feedback (47, 48), existing data indicated that the role of IL-21 in the development of diabetes is more than just an effect on Th17 differentiation. Preclinical studies performed in the NOD mice demonstrate that the IL-21 pathway is critical for disease development (49–51). It acts in a paracrine and autocrine fashion affecting the differentiation and function of several immune cell types in the context of T1D, including $CD4^+$ and $CD8^+$ T cells, NK cells, B cells, macrophages, and dendritic cells (52, 53). Moreover, transgenic overexpression of IL-21 in the pancreatic islets results in autoreactive T cell infiltration and β-cell apoptosis in C57BL/6 mice, a strain free of any kind of autoimmunity signs (54).

As aforementioned, IL-21 is the signature cytokine for Tfh cells, the T lymphocyte subset that is specialized in providing help for B cell antibody production (55). Islet autoantibodies are the best currently available biomarkers to detect ongoing autoimmune process and T1D development risk (56). The production of such antibodies by autoreactive B cells is largely dependent on the function of Tfh cells. By means of an unbiased microarray approach and flow cytometry assay, a recent study assessed T cell differentiation in a mouse model of spontaneous autoimmune diabetes revealing that islet-specific T cells responding to pancreatic antigens show mainly the characteristic features of differentiated Tfh cells (57). Also, adoptive transfer of T cells with a Tfh phenotype from diabetic animals is highly efficient at inducing diabetes to murine recipients. Furthermore, peripheral memory $CD4^+$ T cells from patients with T1D expressed elevated levels of Tfh cell markers (57). In accordance, an increase in peripheral blood Tfh cells has also been reported in three T1D patient independent cohorts, one of which comprised exclusively new-onset patients (58–60).

Interleukin-2 (IL-2) is critical for maintaining the function of the $CD4^+$ regulatory T cells (Tregs), which in turn regulate autoreactive $CD4^+$ effector T cells (Teffs) to prevent autoimmune diseases, such as T1D (61, 62). The involvement of the IL-2 pathway in the physiopathology of T1D first emerged from NOD mice; a reduced IL-2 production by the susceptibility allele (NOD disease-associated SNPs in IL-2 promoter) led to a consequent

reduction of Treg function (63, 64). In humans, certain SNPs of the IL-2 receptor gene, *IL2RA*, encoding the α subunit (CD25) as well as of other genes in the IL-2 pathway, were identified as susceptibility determinants for T1D (65–68). Accordingly, an attenuated IL-2/IL-2R signaling was observed in Treg and Teff cells of T1D patients (69). In a clinical study with recently diagnosed T1D subjects, treatment with low doses of recombinant human IL-2 successfully induced a 10–20% increase in circulating Tregs whereas reduced Teffs, NK cells, and eosinophils (70); these findings lay the groundwork for the potential therapeutic use of rhIL-2 for treating T1D.

At present, emerging evidence suggests that pancreatic-resident Treg subsets have unique effects on the suppression of immune responses in T1D (71). Those distinguishable Treg subpopulations that reside in tissues exhibit special phenotype and function in response to local signals, thereby promoting tissue homeostasis (72). Among those special Treg subsets found in pancreatic tissues and pancreatic lymph nodes involved in preventing inflammation during T1D are: IL-10 secreting $ICOS^+$ Tregs (73, 74), $CXCR3^+$ Tregs (75), and TGF-β-expressing Tregs (76).

In summary, several studies regarding T cell differentiation in T1D clearly demonstrated not only the role of Th1 cells but also the possible involvement of other kind of T-effector cells co-expressing IFN-γ and IL-17, IL-21 producing T cells such as Tfh cells as well as circulating and pancreatic-resident Tregs.

T CELLS CONTRIBUTION TO ADIPOSE TISSUE INFLAMMATION AND OBESITY-ASSOCIATED DIABETES

Type 2 diabetes is a metabolic disease characterized by hyperglycemia resulting from either or both impaired β-cell insulin secretion and increased peripheral insulin resistance; particularly in muscle, liver, and fat (77). The pathogenesis of T2D is complex, it is a multifactorial disease that involves behavioral and environmental factors modulating T2D risk alleles in multiple genes. The pancreatic islets respond to the decrease in insulin-stimulated glucose uptake by enhancing their β-cell mass and insulin secretory activity. When β-cell function can no longer compensate for the prevailing insulin resistance, impaired glucose tolerance and T2D develop.

β-cell dysfunction precedes diabetes, and endoplasmic reticulum (ER) stress contributes to insulin secretory failure. β-cells are particularly susceptible to ER stress due to the high rate of insulin demand in response to rapid changes in glycemia levels. Many environmental factors, including inflammatory cytokines (78), reactive oxygen species (ROS) (79), and viral infections (80), may induce ER stress in β-cells associated with T1D triggering. Dysfunctional β-cells of NOD mice show feature ER stress before overt diabetes (81) and strategies directed to ameliorate ER stress may have therapeutic potential (82). Also, several lines of evidence link inflammation-associated obesity, ER stress, and T2D. The association of ER stress and T2D has been reviewed recently (83).

Inflammation was first linked to insulin resistance and T2D in the early 1990s; an induction of TNF-α expression was systemically and locally observed in adipose tissue from four different rodent models of obesity and diabetes (84). Since then, several studies have described elevated circulating levels of diverse inflammatory factors, such as acute-phase proteins, cytokines, and chemokines in patients with T2D (85–88). Currently, T2D is recognized as a chronic, low-grade inflammatory disease with involvement of pro-inflammatory cytokines and immune cells, including B and T cell subsets as pathogenic mediators (89, 90).

The inflammatory process observed in T2D is usually linked to obesity, a critical risk factor for the disease. Moreover, altered lipolysis in response to over nutrition and rapidly expanding adipose tissue results in elevation of pro-inflammatory saturated free fatty acids (FFAs). FFAs trigger metabolism-associated inflammation through toll-like receptors (TLRs), particularly TLR2 and TLR4, activating signaling pathways that lead to local adipose tissue infiltration by immune cells and systemic insulin resistance (91). The activation of TLR2/4 induces the production of inflammatory cytokines by dendritic cells, macrophages, endothelial cells, and pancreatic islets, as well. During diabetes, high circulating levels of glucose, FFAs, and pro-inflammatory cytokines contribute to insulin resistance and alterations in the immune system (91). Of note, the TLR2/TLR4 expression levels are upregulated in obese individuals (92). Moreover, TLR2- and TLR4-deficient mice are protected from the metabolic undesirable effects of high-fat diet (93) and experiments administering TLR2 antisense-oligonucleotides to high-fat-fed mice recovered insulin sensitivity in adipose tissue (94). Furthermore, nutrient excess may also induce local inflammation in the pancreatic islets (12, 95–97). Tissue inflammation has been detected in pancreatic islets of T2D patients, along with increased levels of cytokines and chemokines. Moreover, all T2D animal models investigated to date display some degree of insulitis (98, 99). TLR2/4 ligands are central in macrophages activation and consequent reduction of insulin secretion from pancreatic β-cells mainly by action of IL-1β and IL-6 on decreased insulin gene expression (100). Also, downstream MyD88-dependent and independent signaling pathways of FFAs-activated TLR2/4 induce differential gene expression and cellular responses leading to islet inflammation and β-cell dysfunction [reviewed in Ref. (101)].

Macrophages are the major immune cell type in adipose tissue, and its relative abundance increased from 5% in lean subjects to a level of up to 50% in obese patients. Moreover, the increase in number is accompanied by an evolution from the anti-inflammatory M2- to the pro-inflammatory M1-phenotype (102); adipose tissue macrophages (ATMs) produce a significant proportion of the inflammatory factors that are upregulated during obesity (95, 96, 103). Therefore, first studies on inflammatory regulation of T2D have been focused on the innate arm of the immune system. However, more recent studies suggest that adaptive immune cells, especially T lymphocytes, generally accumulate in obese adipose tissue in parallel with macrophages and also play a pivotal role in the pathophysiology of T2D (104). Moreover, studies in a mice model of T2D suggest that the accumulation of T lymphocytes in the adipose tissue might occur even before the arrival of macrophages (105).

T cells play a key role during the sequence of events that lead macrophage adipose tissue infiltration. In particular, $CD8^+$ T cells

are activated in adipose tissue which in turn, primer the recruitment and activation of macrophages within this tissue. In fact, infiltration of $CD8^+$ effector ($CD62L^-$ $CD44^+$) T lymphocytes are described as one of the earliest events during the development of adipose tissue inflammation in mice due to obesity caused by *ad libitum* access to a high-fat diet (106). $CD8^+$ T infiltration takes place in obese individuals too, as the expression of *CD8A* in subcutaneous adipose tissue was found elevated in comparison with lean subjects. Interestingly, $CD8^+$ T lymphocytes not only precede adipose tissue infiltration by other immune cells, they are also required for the maintenance of inflammation in obese adipose tissue, since $CD8^+$ T depletion attenuated adipose tissue inflammation and ATMs recruitment, and ameliorated insulin resistance and glucose intolerance in obese mice. $CD8^{-null}$ mice fed a high-fat diet show moderate imbalance of glucose homeostasis. In this respect, gain of function experiments in where $CD8^+$ T cells were administered into obese $CD8^{-null}$ mice aggravate glucose intolerance and insulin resistance, reinforcing the notion that $CD8^+$ T cells are essential for M1 macrophage infiltration and subsequent inflammation in diet-induced obese mice (106).

Visceral adipose tissue (VAT) inflammation involves a complex communication network between different T cell subpopulations expanded by factors that drive differentiation into several kinds of pro-inflammatory effectors. Adipose tissue T cell populations changed with increasing obesity in mice, and an increase in the ratio of $CD8^+$ to $CD4^+$ was reported by various research groups (9, 10, 106, 107). Particular T cell subpopulations play key roles in glucose homeostasis in human and mice. Winer and colleagues reported the importance of VAT resident $CD4^+$ T lymphocytes as modulators of insulin sensitivity in mice under diet-induced obesity; glucose homeostasis was compromised when pathogenic IFN-γ-secreting Th1 cells accumulated in adipose tissue and overwhelmed the static numbers of Th2 and Treg cells. In fact, total absence of INF-γ improved insulin resistance in obese INF-γ KO mice in comparison with control animals having the same diet (108). It was reported that $Rag1^-$ mice, known to be deficient in lymphocytes, developed a T2D phenotype on a high-fat diet, and when adoptively transferred with $CD4^+$ T cells but not $CD8^+$ T cells, normalized glucose tolerance; in particular Th2 signals from the transferred $CD4^+$ T cells were crucial in the protective effect (10). Clinical studies have confirmed the abundant infiltrate of Th1, Th2, and Th17 $CD4^+$ T cells, as well as IFN-γ^+ $CD8^+$ T cells in adipose tissue of healthy overweight and obese humans (109); pro-infammatory Th1, Th17, and IFN-γ^+ $CD8^+$ T cells were markedly increased in VAT relative to subcutaneus adipose tissue. Also, McLaughlin and colleagues confirmed the positive correlation between the relative dominance of Th1 vs Th2 responses in the adipose tissue and peripheral blood and insulin resistance.

A distinctive T cell subpopulation which infiltrates VAT, in a B-lymphocyte dependent way, has been recently identified and resembles senescence-T cells that show up in secondary lymphoid organs with age (110). Phenotypically they are distinguished by expression of $CD44^{hi}CD62L^{lo}CD153^+PD\text{-}1^+$ on the surface of $CD4^+$ T cells and their feature characteristic is the large production of pro-inflammatory osteopontin upon T cell receptor (TCR) stimulation in parallel with compromised IFN-γ and IL-2 secretion. Moreover, they expressed increase senescence associated markers, such as β-gal, γ-H2AX, and *Cdkn1a/Cdkn2b*. This osteopontin-expressing T cells linked visceral adiposity with immune aging (110).

Invariant natural killer T (iNKT) cells are innate T cells involved in inflammatory responses. Adipose tissue-resident iNKT cells protect against obesity and metabolic disorder reducing inflammation in obese individuals (111); they are enriched in human adipose tissue and their number is reduced in obesity (112). iNKT cells express semi-invariant CD1d-restricted TCRs that recognize glycolipid antigens on major histocompatibility complex-like molecule CD1d (113, 114). Huh et al. reported that the absence of CD1d in adipocytes aggravates inflammation in adipose tissue and insulin resistance in obesity suggesting that adipose CD1d is a central activator of adipose iNKT cells. Activated iNKT cells would stimulate counter regulation of inflammation leading to reduced pro-inflammatory responses and insulin resistance in obesity (115).

The relationship between T2D and Th17 cells has also been studied (116). Obesity has been shown to promote expansion of peripheral or adipose tissue-resident IL-17-producing T cells, in human and mice models. In humans, peripheral Th17 cells are increase in T2D patients (117) and positively correlated with body mass index (BMI) but not in metabolically healthy obese subjects (118). Interestingly, T cells from obese T2D donors produced more IL-17 than that from non-diabetic counterparts and this production correlates with T2D severity (118). In diet-induced obese mice an IL-6-dependent expansion of the Th17 T cell pools was observed (119). Specific adipose tissue dendritic cells isolated from obese animals and humans were associated with the differentiation of Th17 cells *in vitro* (120). Studies performed by Zúñiga and colleagues showed an *in vitro* effect of IL-17 on differentiated adipocytes, impairing glucose uptake; *in vivo*, IL-17 deficiency enhanced glucose tolerance and insulin sensitivity in young mice (121).

The role of Treg cells in the maintenance of self-tolerance and the suppression of potentially autoreactive T cells is well known. However, the importance of Treg cells in metabolism has been recognized when it was found that lean adipose tissue enriched in Treg cells (~50% of the $CD4^+$ T cell compartment) controls metabolic status. Indeed, Treg cells in adipose tissue of lean mice provide anti-inflammatory signals to prevent tissue inflammation. Interestingly, Treg cell proportion in the abdominal fat decreases dramatically with obesity (9, 10, 122) resulting in fat tissue inflammation and insulin resistance. Moreover, Feuerer et al. demonstrated that cytokines differentially synthesized by fat-resident Tregs directly affected the synthesis of inflammatory mediators and glucose uptake by cultured adipocytes. Winer et al. associated this Treg mediated protection to the production of IL-10 in ATMs and the restraint of pro-inflammatory macrophage activity, which improves insulin sensitivity.

In accordance, studies in humans showed that the relative proportion of Treg cells in visceral and subcutaneous fat decreased in patients with T2D and negatively correlated with BMI (9, 118) and that there is a decrease in Treg to Th17 and Th1 cell ratios (117). A recent study add complexity to the Treg role on the mechanisms underlying insulin resistance, supporting the concept that

age-associated and obesity-associated IR are driven by distinct adipo-immune populations (123). Bapat and colleagues showed that a particular subset of fat-resident regulatory T cells (fTreg cells) accumulate in VAT as a function of age but not obesity. Additionally, the authors suggest that fTreg cells are functionally distinct from splenic Tregs; while certain canonical genes are similarly expressed, they have discrete expression signatures (i.e., higher expression levels of PPARγ and IL-33 receptor, ST2). Taking advantage of the high expression of ST2 on the surface of fTreg cells, Bapat and co-workers deplete fTreg cells by means of anti-ST2 administration. Interestingly, selective depletion of fTreg cells increases adipose tissue insulin sensitivity implicating these cells as drivers of age-associated insulin resistance (123). Contrary, *in vivo* stimulation of fTreg cells expansion within adipose tissue by treatment with IL-33 decreases insulin sensitivity. All these data suggest that distinct pathophysiologies undergo obesity and age-associated insulin resistance and support the notion that adipo-resident immune cells play a central role in adipose tissue glucose regulation and consequently, whole-body glucose homeostasis in mice.

Interestingly, recent evidences in mice and human suggested that the adipose tissue inflammation associated with obesity, in particular the T cell imbalance, and the impairment in insulin sensitivity, persist even after weight reduction (124, 125). It remains to be elucidated the precise mechanistic pathways of glucose regulation by T cells in human beings.

In summary, the evidence involving the role of T cells in adipose tissue inflammation and insulin resistance suggests that the interplay between T cells, macrophages, and adipocytes is essential. These cells communicate each other in the local adipose tissue environment to activate a sequence of events leading to an inflammatory state. It has been described the role of $CD8^+$ T cells, Th1 and Th17 cells contributing to the obesity-induced insulin resistance phenotype, whereas Th2 cells and Tregs would play a protective role. However, the identity of the trigger that initiates T lymphocyte infiltration within adipose tissue in obesity still remains unknown.

LIVER AND GASTROINTESTINAL RESIDENT T CELLS IN METABOLIC DISORDERS

The liver participates in immunological responses and hepatocytes are also recognized as active immunological mediators among other well-known intrahepatic immune cells (126). There is a subset of innate-like T cells, named mucosal-associated invariant T (MAIT) cells, that recognizes small molecules presented on the non-polymorphic MHC-related protein 1 (MR1) by antigen-presenting cells and express a semi-invariant TCR (127). Like iNKT cells, these non-conventional T cells exhibit restricted TCR diversity recognizing metabolites on MR1 and play a major role in host protection from intracellular pathogens. MAIT cells are scarce in lymphoid tissues, comprising a high proportion of the total intrahepatic and gastrointestinal tract T cells population in humans, having a relevant role as an innate immune barrier against microbial invasion. However, their role in diseases begins to be clarified recently. Interestingly, MAIT cells activate under changes in the composition of gut microbiota and home to inflamed tissues. Magalhaes et al. reported for the first time the existence of MAIT cells abnormalities in severe obese and T2D patients (128). Both, obese and T2D patients showed a decreased in the number of circulating blood MAIT cells as well as dramatic changes in their functionality, i.e., an activated phenotype associated with high Th1- and Th17-type cytokines production. In obese individuals, an elevated number of MAIT cells in inflamed adipose tissue was found suggesting their recruitment from circulation.

Many studies have linked the microbiota, gut integrity, and metabolic disorders. MAIT cells might play a role involving the immune system as a fundamental part of these complex interactions. Recently, Rouxel et al. described that MAIT cells, exhibiting high production of granzyme B and pro-inflammatory cyokines, might directly kill β-cells in humans and NOD mice as well (129). As in the case for T2D patients, a reduced frequency of MAIT cells in peripheral blood of children with recent diagnosis of T1D was described, but not in those who are suffering from the disease for a long period of time. All these evidences highlight the role of MAIT cells in the maintenance of homeostasis within the complex interplay between mucosal integrity and normal islet responses. It would be interesting to investigate the functionality of gastric-resident MAIT cells in gastroparesis, a well-recognized complication of diabetes, since it has been demonstrated a connection between these cells with inflammatory bowel disease (130).

Although the mechanisms triggering and sustaining autoimmunity are not fully understood, the interaction of the intestinal environment with microbiota and, its epithelial integrity play a role in the development of T1D, and the disease in NOD mice (131, 132). A recent paper highlights the relevance of intestinal IL-10-producing type 1 regulatory T (Tr1) cells in the control of Teffs and development of diabetes (133). Increased differentiation of Tr1 cells may account by IL-27 and TGF-β action on intestine. These Tr1 cells have the ability to migrate to islets where they can suppress diabetogenic T cells *via* IL-10 signaling. Moreover, gut microbial metabolites augment the number and function of Treg cells, limiting the frequency of autoreactive T cells and protecting against autoimmune diabetes in NOD mice (134).

SKELETAL MUSCLE RESIDENT T CELLS AND GLUCOSE HOMEOSTASIS

Skeletal muscle is the predominant tissue of insulin-mediated glucose uptake in the postprandial state in humans (135); moreover, lipid accumulation in this tissue is associated with insulin resistance. Muscle insulin resistance is a major factor in the etiology of the metabolic syndrome and T2D (136). The increase in macrophages number within skeletal muscle has been associated to metabolic risk markers and insulin resistance in humans and mice (137, 138). However, little is known about the contribution of T cells infiltration to skeletal muscle inflammation and insulin resistance. Skeletal muscle T cells infiltration occurs in high-fat diet-fed mice (139). T cells localize within skeletal muscle in

intermuscular and perimuscular adipose tissue suggesting that they might play a role in obesity-induced skeletal muscle inflammation and insulin resistance (13). Within skeletal muscle T cells polarized into pro-inflammatory INF-γ-secreting Th1-type inducing myocyte inflammation and insulin resistance through activation of JAK/STAT pathways, while Treg cells diminish in number. Interestingly, $TCRb^{-/-}$ (TCR beta chain null) diet-induced obese mice show reduced skeletal muscle inflammation partially attributable to the lack of Th1 cells, confirming the role of T cells in skeletal muscle inflammation (139). Signals such as chemokines/cytokines/adhesion molecules that induce T cells infiltration into skeletal muscle are not yet identified. However, $CD11a^{-/-}$ mice exhibited low inflammatory gene expression in VAT (140).

Administration of JAK1/JAK2 inhibitors *in vivo* reduces T cells infiltration within skeletal muscle and attenuates insulin resistance (13). Although there is no information, to our knowledge, about the presence of T cells infiltration in skeletal muscle in T1D, it has been described that a particular subpopulation of $CD4^+$ T cells is associated with cachexia in NOD mice (141). In T2D, the level of transcriptome and proteome expression of activated T cells and muscle differ relative to non-diabetic controls (142). T cells, in particular Treg subsets, have homeostatic functions in muscle tissue repair regulating both the inflammatory response, by promoting the switching from M1 to M2 macrophages, and the activation of myogenic stem cells (143). However, further investigation will be required to choose any T lymphocyte subsets as potential targets for improving cachexia in diabetes.

HORMONES, NEUROPEPTIDES, AND NEUROTRANSMITTERS MODULATE T CELL FUNCTION IN DIABETES

T cell capacity to respond against foreign antigens while avoiding reactivity to self-peptides is mainly determined by cellular selection of developing T cells in the thymus (144). Positively selected cells migrate to the peripheral lymphoid organs and target tissues; however, extrathymic pathways of T cell differentiation have also been demonstrated to contributing to the generation of a wide functional spectrum of TCR repertoire.

Several hormones and neurotransmitters impact thymic microenvironment and peripheral tissues affecting T cell development in health and disease (6). In particular, numerous studies performed in human and mice models analyzed the neuroendocrine-immune systems relationship under the metabolic burden of diabetes.

Growth Hormone (GH)—Insulin-Like Growth Factor-1 (IGF-1)

Growth hormone exerts pleiotropic functions modulating from carbohydrate, protein, and fat metabolism to the immune response (145). It is secreted by the anterior pituitary and also produced by immune tissues thereby acting in an autocrine/paracrine manner on immune cells (146).

It was reported that a single point mutation within the DNA binding domain of Stat5b, a central transcription factor downstream GH receptor, is a key molecular defect in NOD mice that limits Foxp3 expression in Treg cells (147, 148). Transgenic NOD mice overexpressing GH show normal glycemia throughout their lives; histochemical analysis of the pancreas revealed the development of peri-insulitis, but showed little or no islet infiltration or β-cell destruction (149). The authors demonstrated that this protective outcome involves several GH-mediated mechanisms on T cells, altering cytokine environment against a Th1 response, maintaining the activity of Treg cell subsets, and affecting Th17/Th1 plasticity. Additionally, sustained GH expression positively influenced β-cell viability.

Conversely, human studies reported that the incidence of T1D during GH replacement therapy in GH-deficient children was comparable with that of the general population (150–152) and described an association of GH treatment with disturbances on carbohydrate metabolism. The hyperglycemic effect of GH has been well-described mainly due to their action on liver, muscle, and adipose tissue (153–155). It is known that many of the GH effects are mediated by the production of IGF-1; thymocytes produce and release IGF-1 and also express its cognate receptor (156).

Several studies propose IGF-1 as a key factor able to induce protection from T1D. Human recombinant IGF-1 administration in NOD mice reduces the severity of insulitis and the incidence of autoimmune diabetes (157–159). The protective T cell-mediated effects of IGF-1 on T1D arose more recently. Anguela and colleagues showed that plasmid-delivered overexpression of IGF-1 in the liver prevents the development of hyperglycemia in a mice model of T1D; decreasing pancreatic infiltration, reducing apoptosis, and increasing replication of β-cell. In this experimental model, they observed an increase of intra-pancreatic Treg cell numbers and proposed an indirect effect mediated by IL-7-producing dendritic cells that improved Treg survival or by the conversion of conventional T cells into Tregs by TGF-β secreted from the liver (160). In a latter study, it was demonstrated that IGF-1 directly stimulates Treg cells proliferation *in vitro* in both mouse and human. Moreover, *in vivo* IGF-1 treatment *via* continuous delivery specifically stimulated proliferation of Treg but no other T cell subtypes and exerted protective action against autoimmune diabetes in two mice models [NOD and multiple low-dose streptozotocin (STZ) injections in C57BL/6J mice] (161). It is noteworthy that the protective effect of IGF-1 treatment might be also exerted at the β-cell level (162–164).

Glucocorticoids (GCs)

Glucocorticoids are endogenous modulators of several biological processes including regulation of metabolism and stress response, and development of the immune system. In particular, GCs broadly affect T cell differentiation and function (165) with positive or negative effects depending on the dose at which they are exposed (166). Synthetic GCs are widely used for their immunosuppressive and anti-inflammatory properties to treat several immune disorders and preventing transplant rejection (167). Brief dexamethasone treatment during acute infection prevents virus-induced autoimmune diabetes in a rat model by down-modulating Th1 responses and restoring the balance between

$CD8^+$ T and Treg cells (168). However, the well-described severe side metabolic effects, such as osteoporosis, hypertension, and insulin resistance, induced by the chronic administration of GCs limits its therapeutic use for autoimmune diabetes (169). It is widely recognized the inhibitory action of GCs, when pharmacologically administered *in vivo*, on the proliferation of several human subpopulation of Ag- and mitogen-stimulated T cells (170). Mechanistically, the underlying inhibitory effects have been attributed to the ability of GCs to restrain gene expression of cytokines. In this respect, IL-2 has been indicated as the principal growth factor for T lymphocyte proliferation (171) However, under physiologic concentrations GCs show contrasting effects promoting TCR-stimulated T cell proliferation (172). CD4 acts as an important coreceptor during Ag recognition by the TCR, contributes to the assembly of TCR-MHC-II complex and thus, increases the sensitivity of T cell to the Ag presented by MHC-II lowering the amount of Ag required to mount an effective immune response. Corticosterone accelerates the expression of CD4 on T cell membrane (173). It has been reported that physiologic concentration of GCs regulates CD4 expression upon T lymphocyte challenge by Concavalin A or TCR stimulation. Also, CD8 expression is induced by GCs on activated mature T cells (174). Therefore, TCR triggering induces the expression of CD4 and CD8 on T lymphocytes and physiologic levels of GCs increase this process enhancing T cell activation.

Glucocorticoids affect gene expression by two main GR-dependent and -independent intracellular mechanisms that exert several biological effects. These differential mechanisms have fueled the interest in the study and development of new GR-ligands with dissociative properties combining GCs' anti-inflammatory properties with a reduced side effect profile (175, 176). These particular dissociated GR-ligands hold potential for their use in Th1-mediated immune disorders. CpdA is a dissociating compound which does not stimulate GR response elements-driven gene expression (177). It has been reported that CpdA regulates T cells through inhibition of the master transcription factor T-bet and induction of GATA-3, thus inhibiting Th1 and favoring Th2 response (178).

In pregnant women at risk of preterm delivery, GCs are routinely administered in order to improve fetal lung development and newborn survival (179). The association of increased exposure to cortisol *in utero* (due to stress, pharmacological treatment, or impaired function of 11β-HSD-2) with long-term effects on glucose-insulin homeostasis has been demonstrated in human and animal models (180–183). However, studies regarding the effects of prenatal GCs on the development of autoimmunity are limited. Recently, using a mice model, Tolosa and colleagues demonstrated that prenatal administration of betamethasone increases apoptosis of developing thymocytes and induces changes in the TCR repertoire decreasing the frequency of pathogenic T cells and protecting from T1D development in NOD mice (184, 185). Conversely, an epidemiological study in Danish cohorts indicated the existence of an increased risk for T1D and T2D in young children who received prenatal steroid treatment (186). Under this scenario, a role of prenatal GCs exposure on pancreas development and T cell effects cannot be ruled out (187).

Ghrelin and Leptin

Peptide hormones known to be involved in the control of eating behavior, glucose metabolism, and energy homeostasis, such as ghrelin and leptin, also exert regulatory effects on the immune system *via* their actions on several leukocytes, including T lymphocytes. Ghrelin and leptin are considered to play mutually antagonistic actions on food intake at the hypothalamic area (188, 189). The interplay between leptin and ghrelin at the level of immune cells was recently recognized. It seems likely in general terms that orexigenic peptides like ghrelin may play a role in promoting endogenous anti-inflammatory responses. On the other hand, anorexigenic agents like leptin might assist inflammation.

Ghrelin is mainly produced by endocrine-like cells in the stomach and released into peripheral blood. Also, the synthesis and secretion of ghrelin by T lymphocytes have been described (190). Human T lymphocytes constitutively express low levels of ghrelin which significantly increase upon cellular activation by stimulated TCR. Moreover, ghrelin enhances proliferation of peripheral $CD4^+$ T cells and thymic murine T cells upon activation with anti-CD3/-CD8 mAbs and during its administration *in vivo*, respectively (191).

It was shown that ghrelin attenuated age-associated and GC-mediated thymic atrophy, and stimulated thymocyte proliferation in young and old mice *in vivo* through activation of its receptor GHS-R1a (191). Thymus involution with age correlates with lower expression levels of intrathymic ghrelin and its receptor, and exogenous administration of ghrelin partially reversed thymus involution and, consequent improvement of thymic progenitors and mature T lymphocytes (192). In addition, ghrelin action on suppressing inflammation might be attributed to the observed inhibition of T derived pro-inflammatory cytokines expression and Th17 development (190, 193). The acylated form of ghrelin exerts potent inhibitory effects on the expression of pro-inflammatory cytokines, such as IL-1β, TNF-α, and IL-6, as well as adhesion molecules by TCR-stimulated T cells. It has been suggested that these inhibitory actions of acylated ghrelin are mediated by GHS-R1a *via* specific blocking of NF-κB and/or STAT3 signaling (190).

There is also evidence that ghrelin is synthesized by T cells and inhibition of its production by using siRNA resulted in stimulation of INF-γ, IL-17 and other chemokines upon TCR ligation indicating that ghrelin might also influence T cell microenvironment regulating immune responses (193). Interestingly, ghrelin downregulates leptin-induced pro-inflammatory Th1 responses (190), suggesting that apart from counteract each other's function at the level of energy homeostasis their interplay might influence T cells function as well. Ghrelin administration delays the development of autoimmune diabetes by reducing islet infiltration in BioBreeding rats; unfortunately, there is absence of information whether this hormone has any effect on diabetogenic T lymphocytes in this setting (194). However, it might be possible the regulation of diabetogenic T cell population through indirect mechanisms such as, an increase in the number or potency of Treg cells due to the reported modulatory effects of ghrelin on monocytes and dendritic cells (190, 195).

Leptin is an adipokine mainly secreted by white adipose tissue, which belongs to the family of the long-chain helical cytokines (IL-2, IL-15, and IL-12) commonly associated with pleiotropic functions. Leptin regulates feeding behavior and metabolism (196), hematopoiesis (197), angiogenesis (198), and reproduction (199). Also, leptin exerts modulatory actions on the immune systems (200). It was shown that leptin induces proliferation and secretion of IL-2 by $CD4^+$ T lymphocytes in humans and mice (201). In addition, leptin assists Th1 cell-biased immune responses stimulating the secretion of INF-γ by T cells (202). Therefore, leptin promotes pro-inflammatory immune responses like the antigen-specific Th1-type directed against β cells observed in T1D. In fact, it has been reported that administration of leptin during early life accelerates the development of autoimmune diabetes in the NOD mice (203). Interestingly, Materese et al. found that circulating leptin peaked soon before the onset of hyperglycemia and spontaneous diabetes in female prone NOD mice. The administration of leptin enhanced the production of IFN-γ by peripheral T lymphocytes. On the other hand, a mutated version of the leptin-receptor in NOD mice suppresses autoimmune diabetes progression (204). All these evidences point leptin with its permissive action on the development of polarized Th1-type autoimmunity against β cells.

Insulin

Only sparse data are available regarding the role of insulin on T lymphocytes. It has been reported that insulin infusion resulted in reduction of NF-κB and ROS generation, and increase in IκB in mononuclear cells, all changes characteristic of an anti-inflammatory effect at the molecular level (205). Unfortunately, this study did not address whether there is a similar response to insulin treatment in all mononuclear cells or there is a particular cellular type more sensible to insulin action. Later, it was elucidated that insulin drives T cell differentiation toward an anti-inflammatory Th2-phenotype by mechanisms that involve ERK activation (206). Nevertheless, other study found that in T cells isolated from obese subjects incubation with supra-physiological concentration of insulin did not alter the Th1/Th2 balance suggesting that insulin signaling in lymphocytes is strongly impaired in obesity, shifting T-cell differentiation toward a pro-inflammatory phenotype (207). During diabetes there is a high occurrence of apoptosis in lymphocytes and insulin treatment reduces this effect, suggesting that insulin may act as a pro-survival factor for lymphocytes (208). Moreover, there is evidence in favor of a role of insulin in promoting obesity-associated adipose tissue inflammation (209).

A recent theoretical study simulated how hyperinsulinemia might alter the dynamics of the $CD4^+$ T regulatory network (210); the analysis showed how high insulin levels affect the differentiation and plasticity of $CD4^+$ T cells favoring stabilization of inflammatory Th1 and Th17 and reducing the stability of Treg types. In line with this *in silico* observations, it has been demonstrated *in vitro* that Tregs express the insulin-receptor and that high levels of insulin specifically inhibits IL-10 production *via* AKT/mTOR signaling and impairs the ability of Treg cells to suppress TNF-α production by macrophages (211). Moreover, the authors showed that Tregs from the VAT of hyperinsulinemic diet-induced obese mice exhibited a specific decrease in IL-10 production, as well as a parallel increase in IFN-γ production; suggesting that hyperinsulinemia may contribute to the development of obesity-associated inflammation *via* modulation of Treg function.

Resting T lymphocytes do not express detectable levels of insulin-receptor; however, after activation its expression is significantly increased (206, 212, 213). A more recent study suggests that upregulation of the insulin-receptor on activated T cells is critical for T cell function and efficient adaptive immune response (214). In conditions of impaired insulin-receptor expression, T-effector activities are diminished resulting in attenuated clinical symptoms in a T-cell-mediated multiple sclerosis model *in vivo* (214). Fischer et al. showed that silencing the insulin-receptor on T lymphocytes disrupts their function, such as reducing cytokine production, proliferation, and migration without affecting thymocytes development. Interestingly, the absence of insulin-receptor affected $CD4^+$ and $CD8^+$ T subsets whereas the frequency and potency of Treg cells were unaffected (214).

T lymphocytes use aerobic glycolysis (Warburg effect) upon activation and their increase in glucose demand is facilitated by induction of the insulin-receptor along with GLUT1 (215). Given the critical dependence on glucose upon activation, glycemic status should be considered as a factor affecting T cell function. The diabetic state, where circulating glucose levels are elevated, provides an environment of oxidative stress and activation of the inflammatory pathways. Transgenic expression of Glut1 augmented T cell activation and led to accumulation of readily activated memory-phenotype T cells with signs of autoimmunity in aged mice (216). Increased glucose uptake may lead to excessive T cell activity and accumulation as a result of enhanced T cell activation and/or inhibition of T cell death following stimulation. Moreover, human $CD4^+$ and $CD8^+$ T cells differ in the relative use of the metabolic pathways contributing to functional responses. Thus, $CD4^+$ T subset shows higher basal glycolysis mainly attributed to elevated expression of glycolytic enzymes and $CD8^+$ T subpopulation showing a decrease in glycolysis upon activation and greater dependency on mitochondrial metabolism for cytokine production. Also, it was demonstrated that the binding affinity of specific antigens fine-tune T cell metabolism (217). Therefore, T lymphocyte insulin-receptor/GLUTs expression, insulin and glucose levels as well as, the affinity of antigens with cognate TCR of different T cell subsets all have implications to consider for therapeutic manipulation in the setting of hyperglycemia and hyperinsulinemia (T2D) and, during T-cell-mediated T1D featured by elevated glycemia and lack of insufficient insulin levels.

Prolactin (PRL)

Prolactin is a pituitary hormone not only essential for reproduction and lactation but also involved in immunological responses. PRL and its receptor are expressed by various extra-pituitary tissues, including lymphoid cells (218, 219). PRL has a stimulatory action on the immune system; it affects differentiation and maturation of both, B and T lymphocytes, stimulates lymphocyte proliferation and macrophage function, and enhances inflammatory responses and production of immunoglobulins (220–222).

Increase serum PRL has been detected in autoimmune disorders including T1D and elevated prolactinemia was also

observed in T2D (223–225). The association between circulating PRL levels and glucose homeostasis has been controversial. Within the physiological range, higher serum PRL levels seem to be associated with insulin resistance in men (226) and with reduced glucose tolerance in the third trimester of pregnancy in women (227). Conversely, higher circulating PRL levels were associated with lower prevalence of diabetes and impaired glucose regulation in a large cohort of middle-aged and elderly men and postmenopausal women (228).

Experimental studies suggested a protective role associated with PRL modulation of T cell development; PRL reduces insulitis and protects against autoimmune diabetes in NOD mice (229) in the autoinmune diabetes model induced by low-dose STZ administration in C57BL/6 mice (230). Further studies in this latter experimental model showed that PRL treatment enhances a Th2 response by increasing the frequency of IL-10 positive splenocytes and down-modulating the featured expression of the Th1 cytokines IFN-γ and TNF-α in splenocytes (231). Furthermore, PRL-expanded Treg ($CD4^+$ $Foxp3^+$) population and improved the efficacy of short-term low-dose anti-CD3 treatment (which induce a transient $CD4^+$ and $CD8^+$ T cell depletion) at achieving diabetes remission in the NOD mice (232). Conversely, severe hyperprolactinemia induced by anterior pituitary ectopic transplantation increases the incidence of diabetes in the NOD mice (233). A study analyzing the *in vitro* effect of PRL on $CD4^+$ T cell suggested that the modulatory effect is dose dependent; low-dose PRL promotes Th1 response through increases in its Th1-driven transcription factor T-bet, whereas higher doses have suppressive effects (234). Therefore, differences obtained in clinical and experimental studies might be explained on the basis of the PRL differential effect on T cells, glucose metabolism, and insulin resistance depending of the hormone concentration impacting on target tissues.

Moreover, it was demonstrated that PRL stimulates insulin secretion and proliferation of β-cells in murine and human islets (235–237) and in particular during pregnancy (238). Thus, a further protective action of PRL exerted at β-cells level could not be ruled out in the experimental models studied.

Oxytocin (OXT)

Oxytocin is an essential neuropeptide involved in the regulation of maternal behavior, lactation, and parturition (239). In the central nervous system OXT is expressed in subpopulations of hypothalamic neurons, stored in the neurohypophysis and released into circulation. Besides its central origin, OXT is produced and released in peripheral tissues acting in a paracrine and autocrine fashion *via* widely expressed OXT receptors (240). In addition to the abovementioned physiological functions in mammals, the modulatory effect of the OXT-secreting system on immune system activity and metabolic homeostasis has come to gain attention.

Oxytocin effects on immune functions include thymus physiology, immunologic defense, homeostasis, and surveillance (241). However, scarce information exists regarding the interaction of OXT with T lymphocytes in diabetes. CD38, a membrane ADP-ribosyl cyclase expressed in several cells such as lymphocytes and β-cells, is involved in OXT secretion (242); targeted disruption of CD38 accelerates autoimmune diabetes in NOD mice by enhancing autoimmunity (243). CD38-deficient mice presented a disbalance between T-effector and Treg cells and an age-dependent increase in a diabetogenic CD8 clonotype, along with impaired insulin secretion and an elevated plasma glucose level.

Recent studies have shown that the impairment of OXT signaling is associated with disturbance of metabolic homeostasis, resulting in obesity and diabetes. In mice under a high-fat diet, there was a significant increase in both OXT and OXT receptor levels in the brain, as well as an increase in OXT receptor in the islets (244). OXT receptor-deficient mice exhibited increase β-cell death under metabolic stress conditions resulting in impaired insulin secretion and glucose intolerance under a high-fat diet (244). Both OXT- and OXT receptor-deficient mice developed late-onset obesity (245, 246).

On the other hand, peripheral OXT treatment improved glucose tolerance and reduced food intake and visceral fat mass in mice under diet-induced obesity (247, 248). Moreover, OXT treatment improved glucose homeostasis and induced tissue regenerative changes of pancreatic islets after STZ-induced diabetes in rats (249); similar results were obtained in mice (248). Conversely, worsening of basal glycemia and glucose tolerance were observed under OXT treatment in ob/ob animals (250) suggesting that OXT effects on glucose metabolism may depend on the interaction with leptin signaling.

A central action of OXT on glucose homeostasis was also observed. Intranasal OXT delivery enhanced glucose tolerance and β-cell response in healthy men challenged with an oral glucose tolerance test (251). Furthermore, OXT nasal spray treatment in obese patients effectively reversed obesity and related lipid disorders and improved blood glucose and insulin postprandial levels (248). In addition, third-ventricle injections of OXT improved glucose intolerance and fasting blood insulin levels in mice under chronic high-fat diet feeding and led to significant improvements in glucose tolerance, β-cell insulin secretion, and blood insulin levels in the multiple low-doses administration of STZ-induced autoimmune diabetes in mice (248).

Sexual Steroids

For most of autoimmune diseases, females are generally more frequently affected than males. This is the case for systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis. However, sexual dimorphism in autoimmune diabetes prevalence is observed in NOD mice but not in humans (252). One of the main factors contributing to gender differences in immune system is sex hormones. The effects exerted by female (estrogen, progesterone) and male (androgens) steroid hormones on T lymphocytes might explain gender differences in specific autoimmune diseases (253).

Several studies indicate that testosterone has suppressive effects on T cells by inhibiting Th1 differentiation of naive $CD4^+$ T cells and pro-inflammatory cytokine production and enhancing the expression of anti-inflammatory cytokines (254, 255). Ovarian hormones also modulate T lymphocyte function. *In vivo* and *in vitro* evidence indicate that progesterone, which promotes maternal–fetal tolerance during pregnancy, favors the Th2, and suppresses Th1 and Th17 responses, and has a potent

Treg induction activity promoting the production of anti-inflammatory cytokines like TGF-β1 and IL-10 (256, 257). Numerous evidences support estrogens influence on the development and maintenance of thymic and peripheral T cell function with dual effects depending on factors, such as steroid concentration, target cell, and timing (258). Estradiol at periovulatory to pregnancy levels stimulates IL-4 and IL-10 production and inhibits TNF-α from $CD4^+$ T cells and increases Th2 and Treg phenotype, which might shift the immune response toward tolerance (258, 259). On the other hand, at lower concentrations, estradiol stimulates TNF-α, IFN-γ, and IL-1β production (258, 260). Scarce information is available regarding sexual steroids and T cell interaction under the burden of diabetes.

NOD mice spontaneously develop diabetes with a strong female prevalence; a more invasive and destructive insulitis, leading to an earlier onset and higher incidence is observed in females (261). Moreover, the incidence of diabetes was significantly decreased in female NOD mice, but increased in male, by castration at the time of weaning (262, 263). Furthermore, long-term administration of androgen or its derivatives to young female NOD mice resulted in a decrease in the percentage of $CD4^+$ T cells in peripheral blood mononuclear cells and the incidence of diabetes (264, 265). Bao and colleagues demonstrated that sex hormones modulate the Th1/Th2 balance in the early stages of the T cell-mediated autoimmune process in the NOD mice; IFN-γ expression was significantly higher in pancreatic and lymph node-T cells from young females, whereas IL-4 expression was higher in male counterparts. This differential expression, enhancing Th1 immune response in female NOD mice, was found to be due to the upregulation of IL-12 induced IFN-γ production through activation of STAT4 by estrogen (266). Additionally, it was suggested that male-specific gut microbiome play a protective role in NOD mice that is mediated, at least in part, *via* microbiota metabolism of sex hormones (267). Conversely, estradiol administration was found to restore immunomodulatory functions of iNKT cells and preserve female NOD mice from both spontaneous and cyclophosphamide-induced diabetes (268).

A clear sexual dimorphism is observed related to glucose metabolism and obesity-associated T2D. The sex difference in the prevalence of diabetes was reversed during reproductive life, there are more men with T2D at middle age while there are more affected women after menopause (269), suggesting a protective role of estrogens. Consistent with this observation, continuous estradiol treatment (pregnancy levels) in males inhibited weight gain and the associated onset of hyperglycemia in an islet amyloid (huIAPP)-dependent murine model of diabetes; histological analysis of the pancreas revealed estradiol prevented deposition of islet amyloid and preserved islet mass and β-cells insulin content (270). Mice of both sexes develop a vulnerability to STZ-induced insulin deficiency when estradiol production or signaling is genetically suppressed (aromatase-deficient, $ArKO^{-/-}$ and ERα-deficient, $ERKO^{-/-}$ mice); in these mice, estradiol treatment prevents STZ-induced β-cell death and helps sustain insulin production, and prevents diabetes (271). Estradiol protective effect on β-cells was also observed in isolated human pancreatic islets; estradiol treatment of cytokine-challenged islets increases islet viability by lowering NF-κB activity and caspase-9 activation and cytokine-induced cell death. Additionally, estradiol improved glucose-stimulated insulin response *in vitro* and *in vivo* functionality of treated human islets after transplantation in the portal vein of STZ-induced NOD*scid* mice (272).

Estrogen protective action on glucose homeostasis is not only exerted in the pancreas; several studies indicated that estradiol enhances insulin sensitivity in peripheral tissues, improves body fat distribution, and reduces adipose tissue inflammation (273–275). Estrogen treatment prevented insulin insensitivity and reduced the expression of adipose tissue inflammation (*Mcp-1* and *Cd68*) induced by high-fat diet in ovariectomized mice (274).

Although its protective anti-inflammatory effect on immune cells, progesterone has been associated with the development of gestational diabetes. It was demonstrated that the hyperglycemic effect of gestational levels of progesterone is mostly due to the enhancement of insulin resistance (276), particularly by a reduction of glucose transporter 4 expression in skeletal muscle and adipose tissue (277) but also reducing insulin secretion by a non-genomic mechanism (278). A recent study performed in RINm5F β-cell line and primary rat islets show that progesterone, particularly at pharmacological concentrations used for preterm delivery prevention, induced apoptosis of pancreatic β-cells through an oxidative-stress-dependent mechanism (279), contributing to gestational diabetes pathogenesis.

It is well established the impact of testosterone deficiency on the development of visceral obesity and insulin resistance in men (280, 281). Consistently, androgen receptor-deficient mice exacerbates adiposity and insulin resistance induced by a high-fat diet; elevated serum IL-1β levels and decreased pancreatic glucose-stimulated insulin secretion was also observed (282). A recent transcriptome analysis of islets from adult male mice lacking androgen receptor selectively in β-cells revealed alterations in genes involved in inflammation and β-cell function (283).

Recently, Rubinow and colleagues analyzed lymphocyte subsets in subcutaneous adipose tissue biopsies after 4 weeks of pharmacological testosterone suppression with a GnRH receptor antagonist and controlled testosterone replacement in healthy male subjects. In this clinical study, change in serum total testosterone levels correlated inversely with $CD3^+$, $CD4^+$, and $CD8^+$ T cells and ATMs within adipose tissue (275).

At the pancreas level, it was observed a sex specific protective action of testosterone on STZ-induced apoptosis in β-cells; the cytoprotective effect was seen in gonadectomized male but not in female rats (284, 285). Moreover, chronic hyperandrogenism induced β-cell dysfunction and failure to compensate high-fat diet induce insulin resistance in female mice (286). The sexual dimorphism in the modulation of glucose and energy homeostasis by testosterone is evidenced in the clinic, androgen excess predisposes to insulin resistance, β-cell dysfunction, and T2D in women (281). Nonetheless, further research is needed to reveal the mechanisms underlying the sex differences in the metabolic effect of testosterone.

Neurotransmitters

Originally, the notion that neurotransmitters act as immunomodulators emerged with the discovery that their release from the nervous system could lead to signaling through lymphocyte

cell-surface receptors modulating immune response. It is now known that neurotransmitters can also be released from immune cells and act as autocrine or paracrine modulators.

It has been demonstrated that administration of gamma-aminobutyric acid (GABA), a major CNS neurotransmitter synthesized from glutamate by glutamic acid decarboxylase (GAD), exerts antidiabetic effects by acting on both islet β-cells and the immune system in both T1D and T2D models. GABA acts as an autocrine excitatory neurotransmitter in human pancreatic β-cells through GABA receptors (287, 288).

Gamma-aminobutyric acid promotes proliferation, protects β-cells from STZ- and cytokine-induced apoptosis (288), and inhibits human β-cell apoptosis following islet transplantation into NOD*scid* mice (289). This protective effect is also observed *in vivo*, e.g., GABA treatment prevents insulitis and diabetes onset and preserves insulin expression in NOD mice and in multiple low-dose STZ-induced diabetes in C57BL/6 mice (288, 290, 291) and delays hyperglycemia in the adoptive transfer of disease in NODscid mice (292). Moreover, overtly diabetic NOD mice treated with GABA improved fasting glycemia, insulin and C-peptide levels and glucose tolerance (291).

Also, GABA receptors are expressed in various immune cells, including T cells (292, 293). Low doses of GABA inhibited activated T cell responses against islet autoantigens when assayed *ex vivo* (292), suggesting that GABA downregulates diabetogenic Teff function *in vivo*. Later studies showed an anti-inflammatory effect of GABA treatment, increasing the frequency and suppressive activity of splenic $CD4^+Foxp3^+$ Tregs in pancreatic lymph nodes in NOD mice with no changes in GAD-reactive $CD4^+$ T cells and decreased circulating inflammatory cytokines in the multiple low-dose STZ-induced diabetes model (288, 291).

A beneficial effect of GABA was observed also in T2D experimental models. Oral GABA administration inhibited obesity, reduced fasting blood glucose, and improved glucose tolerance and

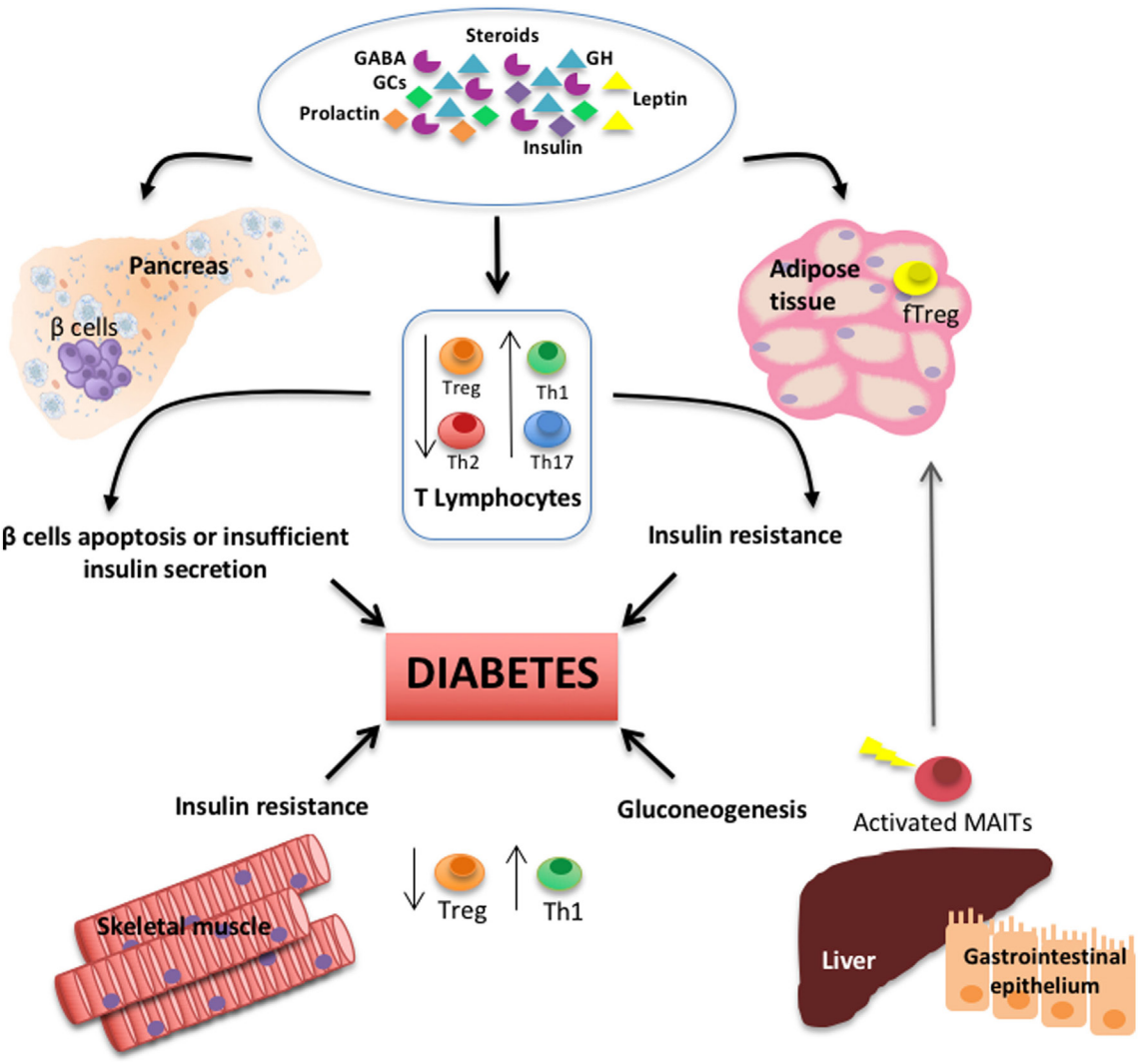

FIGURE 1 | Schematic diagram depicts the interplay among T lymphocyte subsets, several types of hormones and neurotransmitters, and primary peripheral tissues regulating glucose metabolism and during the pathogenesis of diabetes.

insulin sensitivity in high-fat diet-fed C57BL/6 mice. Moreover, even after the onset of obesity and hyperglycemia, GABA treatment improved glucose homeostasis (294). Furthermore, GABA treatment inhibited obesity-related inflammation, reducing the frequency of VAT macrophage infiltrates and increasing the frequency of splenic $CD4^+Foxp3^+$ Tregs in high-fat diet-fed mice (294).

In accordance with the antidiabetic effect in preclinical models, GABA and GABA analogs were also shown to exert insulinotropic effects in humans (295, 296).

Interestingly, consistent with the high levels of GAD found in the islets of Langerhans, GAD65 is one of the major target autoantigens recognized by self-reactive T cells in T1D. Complete suppression of β-cell GAD expression in NOD mice blocked the generation of diabetogenic T cells, protected islet grafts from autoimmune injury and consequently, the development of autoimmune diabetes (297). In fact, potential immunomodulation with GAD therapy has been extensively investigated for the prevention or treatment of T1D in humans (298).

Histamine is an inflammatory mediator classically involved in allergic reactions but also in the modulation of innate immunity and autoimmune reactions. Its diverse effects are mediated by the differential expression and regulation of four known histamine receptors (termed H1R-H4R) and their distinct intracellular signals (299). Th1 and Treg cells express relatively high levels of H1R, whereas H2R is preferentially expressed by Th2 cells. Histamine modulates T lymphocytes by enhancing Th1 responses through H1R and downregulates both the 1- and 2-type responses through H2R (300); activation of H1R by histamine decreases Treg cell suppressive functions.

The association of autoimmune diseases, such as multiple sclerosis, rheumatoid arthritis, and diabetes, and elevated serum and tissue histamine levels was described many years ago (301–303). However, research searching for the possible role of histamine signaling on diabetes emerged recently.

In histidine decarboxylase (HDC) deficient NOD mice, the lack of endogenous histamine reduces IL-12 and IFN-γ levels and delays the onset of autoimmune diabetes (304); the proportion $CD4^+CD25^+Foxp3^+$ Treg cells in spleen and pancreatic lymph node remained unchanged. Surprisingly, exogenous histamine administration not only failed to increase the incidence of T1D but also delayed the onset of disease in both wild-type and $HDC^{-/-}$ mice (304).

Central histamine signaling is involved in the control of feeding behavior and energy homeostasis. H3R is principally expressed in histamine neurons and negatively regulates the synthesis and release of histamine. Treatment with a H3R agonist decreases appetite, body weight, and insulin resistance in diet-induced obese mice (305). On the other hand, targeted disruption of H3Rs leads to an obese phenotype (306). Moreover, mice deficient in histamine H1R or HDC showed a dysregulation in the leptin signaling, impaired glucose tolerance, and are prone to become obese on a high-fat diet or at advanced age (307–309).

It was recently reported that the H1R antagonist cetirizine partially counteracts cytokine- and oxidative stress-induced β-cell death (310). *In vivo*, H1R antagonist ameliorates high-fat diet-induced glucose intolerance in male C57BL/6 mice, but no effect was observed on diabetes outcome in female NOD mice, suggesting a protective effect of cetirizine against high-fat diet-induced β-cell dysfunction, but not against autoimmune β-cell destruction (311).

CONCLUSION

T lymphocytes, as important components of the adaptive arm of the immune system, are key players in the modulation of metabolism in several tissues in health and disease (see **Figure 1**). The neuroendocrine system plays an essential role controlling the number and activity of different T cell subpopulations. Herein, we collected data that warrant further investigation on T lymphocytes biology hoping that it would lay the groundwork for future translational research that aims to restore homeostasis in metabolic disorders and treat diabetes in its multiple forms.

AUTHOR CONTRIBUTIONS

LA and MP contributed to the conception and design of the review article and wrote sections of the manuscript; MG created the model figure. All authors contributed to manuscript revision, read and approved the submitted version.

FUNDING

The present work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET)-Argentina and FOCEM-Mercosur Grant COF 03/11 to MP.

REFERENCES

1. Besedovsky H, del Rey A, Sorkin E, Da Prada M, Burri R, Honegger C. The immune response evokes changes in brain noradrenergic neurons. *Science* (1983) 221(4610):564–6. doi:10.1126/science.6867729
2. Besedovsky H, del Rey A, Sorkin E, Dinarello CA. Immunoregulatory feedback between interleukin-1 and glucocorticoid hormones. *Science* (1986) 233:652–4. doi:10.1126/science.3014662
3. Blalock JE, Smith EM, Meyer WJ III. The pituitary-adrenocortical axis and the immune system. *Clin Endocrinol Metab* (1985) 14:1021–38. doi:10.1016/S0300-595X(85)80087-6
4. Smith EM, Morrill AC, Meyer WJ III, Blalock JE. Corticotropin releasing factor induction of leukocyte-derived immunoreactive ACTH and endorphins. *Nature* (1986) 321(6073):881–2. doi:10.1038/321881a0
5. Steinman L. Elaborate interactions between the immune and nervous systems. *Nat Immunol* (2004) 5:575–81. doi:10.1038/ni1078
6. Savino W, Mendes-da-Cruz DA, Lepletier A, Dardenne M. Hormonal control of T-cell development in health and disease. *Nat Rev Endocrinol* (2016) 12(2):77–89. doi:10.1038/nrendo.2015.168
7. Hotamisligil GS. Inflammation and metabolic disorders. *Nature* (2006) 444:860–7. doi:10.1038/nature05485
8. Zhang X, Zhang G, Zhang H, Karin M, Bai H, Cai D. Hypothalamic IKKβ/NF-κB and ER stress link overnutrition to energy imbalance and obesity. *Cell* (2008) 135:61–73. doi:10.1016/j.cell.2008.07.043
9. Feuerer M, Herrero L, Cipolletta D, Naaz A, Wong J, Nayer A, et al. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nat Med* (2009) 15(8):930–9. doi:10.1038/nm.2002

10. Winer S, Chan Y, Paltser G, Truong D, Tsui H, Bahrami J, et al. Normalization of obesity-associated insulin resistance through immunotherapy. *Nat Med* (2009) 15:921–9. doi:10.1038/nm.2001
11. Park EJ, Lee JH, Yu GY, He G, Ali SR, Holzer RG, et al. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. *Cell* (2010) 140:197–208. doi:10.1016/j.cell.2009.12.052
12. Sell H, Habich C, Eckel J. Adaptive immunity in obesity and insulin resistance. *Nat Rev Endocrinol* (2012) 8(12):709–16. doi:10.1038/nrendo.2012.114
13. Khan IM, Perrard XY, Brunner G, Lui H, Sparks LM, Smith SR, et al. Intermuscular and perimuscular fat expansion in obesity correlates with skeletal muscle T cell and macrophage infiltration and insulin resistance. *Int J Obes (Lond)* (2015) 39(11):1607–18. doi:10.1038/ijo.2015.104
14. International Diabetes Federation. *IDF Diabetes Atlas*. 8th ed. Brussels, Belgium: International Diabetes Federation (2017). Available from: http://www.diabetesatlas.org
15. Willcox A, Richardson SJ, Bone AJ, Foulis AK, Morgan NG. Analysis of islet inflammation in human type 1 diabetes. *Clin Exp Immunol* (2009) 155(2):173–81. doi:10.1111/j.1365-2249.2008.03860.x
16. Campbell-Thompson M, Fu A, Kaddis JS, Wasserfall C, Schatz DA, Pugliese A, et al. Insulitis and β-cell mass in the natural history of type 1 diabetes. *Diabetes* (2016) 65(3):719–31. doi:10.2337/db15-0779
17. Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. *Lancet* (2014) 383(9911):69–82. doi:10.1016/S0140-6736(13)60591-7
18. Cudworth AG, Woodrow JC. Letter: HL-A antigens and diabetes mellitus. *Lancet* (1974) 2:1153. doi:10.1016/S0140-6736(74)90930-1
19. Noble JA, Valdes AM. Genetics of the HLA region in the prediction of type 1 diabetes. *Curr Diab Rep* (2011) 11(6):533–42. doi:10.1007/s11892-011-0223-x
20. Bakay M, Pandey R, Hakonarson H. Genes involved in type 1 diabetes: an update. *Genes (Basel)* (2013) 4(3):499–521. doi:10.3390/genes4030499
21. Redondo MJ, Steck AK, Pugliese A. Genetics of type 1 diabetes. *Pediatr Diabetes* (2017) 19(3):346–53. doi:10.1111/pedi.12597
22. Sarvetnick N, Liggitt D, Pitts SL, Hansen SE, Stewart TA. Insulin-dependent diabetes mellitus induced in transgenic mice by ectopic expression of class II MHC and interferon-gamma. *Cell* (1988) 52:773–82. doi:10.1016/0092-8674(88)90414-X
23. Rapoport MJ, Jaramillo A, Zipris D, Lazarus AH, Serreze DV, Leiter EH, et al. Interleukin 4 reverses T cell proliferative unresponsiveness and prevents the onset of diabetes in nonobese diabetic mice. *J Exp Med* (1993) 178:87–99. doi:10.1084/jem.178.1.87
24. Rabinovitch A. Immunoregulatory and cytokine imbalances in the pathogenesis of IDDM. Therapeutic intervention by immunostimulation? *Diabetes* (1994) 43:613–21. doi:10.2337/diab.43.5.613
25. Katz JD, Benoist C, Mathis D. T helper cell subsets in insulin-dependent diabetes. *Science* (1995) 268:1185–8. doi:10.1126/science.7761837
26. Savinov AY, Wong FS, Chervonsky AV. IFN-gamma affects homing of diabetogenic T cells. *J Immunol* (2001) 167:6637–43. doi:10.4049/jimmunol.167.11.6637
27. Arif S, Tree TI, Astill TP, Tremble JM, Bishop AJ, Dayan CM, et al. Autoreactive T cell responses show pro-inflammatory polarization in diabetes but a regulatory phenotype in health. *J Clin Invest* (2004) 113:451–63. doi:10.1172/JCI19585
28. Han D, Leyva CA, Matheson D, Mineo D, Messinger S, Blomberg BB, et al. Immune profiling by multiple gene expression analysis in patients at-risk and with type 1 diabetes. *Clin Immunol* (2011) 139:290–301. doi:10.1016/j.clim.2011.02.016
29. Zaccone P, Burton OT, Gibbs SE, Miller N, Jones FM, Schramm G, et al. The *S. mansoni* glycoprotein ω-1 induces Foxp3 expression in NOD mouse $CD4^+$ T cells. *Eur J Immunol* (2011) 41(9):2709–18. doi:10.1002/eji.201141429
30. Walker LS, von Herrath M. CD4 T cell differentiation in type 1 diabetes. *Clin Exp Immunol* (2016) 183(1):16–29. doi:10.1111/cei.12672
31. Arif S, Gibson VB, Nguyen V, Bingley PJ, Todd JA, Guy C, et al. β-cell specific T-lymphocyte response has a distinct inflammatory phenotype in children with Type 1 diabetes compared with adults. *Diabet Med* (2017) 34(3):419–25. doi:10.1111/dme.13153
32. Vukkadapu SS, Belli JM, Ishii K, Jegga AG, Hutton JJ, Aronow BJ, et al. Dynamic interaction between T cell-mediated beta-cell damage and beta-cell repair in the run up to autoimmune diabetes of the NOD mouse. *Physiol Genomics* (2005) 21:201–11. doi:10.1152/physiolgenomics.00173.2004
33. Perone MJ, Bertera S, Shufesky WJ, Divito SJ, Montecalvo A, Mathers AR, et al. Suppression of autoimmune diabetes by soluble galectin-1. *J Immunol* (2009) 182(5):2641–53. doi:10.4049/jimmunol.0800839
34. Bending D, De la Peña H, Veldhoen M, Phillips JM, Uyttenhove C, Stockinger B, et al. Highly purified Th17 cells from BDC2.5NOD mice convert into Th1-like cells in NOD/SCID recipient mice. *J Clin Invest* (2009) 119(3):565–72. doi:10.1172/JCI37865
35. Martin-Orozco N, Chung Y, Chang SH, Wang YH, Dong C. Th17 cells promote pancreatic inflammation but only induce diabetes efficiently in lymphopenic hosts after conversion into Th1 cells. *Eur J Immunol* (2009) 39:216–24. doi:10.1002/eji.200838475
36. Honkanen J, Nieminen JK, Gao R, Luopajarvi K, Salo HM, Ilonen J, et al. IL-17 immunity in human type 1 diabetes. *J Immunol* (2010) 185:1959–67. doi:10.4049/jimmunol.1000788
37. Liu SM, Lee DH, Sullivan JM, Chung D, Jäger A, Shum BO, et al. Differential IL-21 signaling in APCs leads to disparate Th17 differentiation in diabetes-susceptible NOD and diabetes-resistant NOD.Idd3 mice. *J Clin Invest* (2011) 121:4303–10. doi:10.1172/JCI46187
38. Li CR, Mueller EE, Bradley LM. Islet antigen-specific Th17 cells can induce TNF-alpha-dependent autoimmune diabetes. *J Immunol* (2014) 192:1425–32. doi:10.4049/jimmunol.1301742
39. Joseph J, Bittner S, Kaiser FM, Wiendl H, Kissler S. IL-17 silencing does not protect nonobese diabetic mice from autoimmune diabetes. *J Immunol* (2012) 188:216–21. doi:10.4049/jimmunol.1101215
40. Marwaha AK, Crome SQ, Panagiotopoulos C, Berg KB, Qin H, Ouyang Q, et al. Cutting edge: increased IL-17-secreting T cells in children with new-onset type 1 diabetes. *J Immunol* (2010) 185:3814–8. doi:10.4049/jimmunol.1001860
41. Arif S, Moore F, Marks K, Bouckenooghe T, Dayan CM, Planas R, et al. Peripheral and islet interleukin-17 pathway activation characterizes human autoimmune diabetes and promotes cytokine-mediated beta-cell death. *Diabetes* (2011) 60:2112–9. doi:10.2337/db10-1643
42. Reinert-Hartwall L, Honkanen J, Salo HM, Nieminen JK, Luopajärvi K, Härkönen T, et al. Th1/Th17 plasticity is a marker of advanced beta cell autoimmunity and impaired glucose tolerance in humans. *J Immunol* (2015) 194:68–75. doi:10.4049/jimmunol.1401653
43. Mandrup-Poulsen T, Bendtzen K, Nerup J, Dinarello CA, Svenson M, Nielsen JH. Affinity-purified human interleukin I is cytotoxic to isolated islets of Langerhans. *Diabetologia* (1986) 29:63–7. doi:10.1007/BF02427283
44. Eizirik DL, Colli ML, Ortis F. The role of inflammation in insulitis and β-cell loss in type 1 diabetes. *Nat Rev Endocrinol* (2009) 5:219–26. doi:10.1038/nrendo.2009.21
45. Grieco FA, Moore F, Vigneron F, Santin I, Villate O, Marselli L, et al. IL-17A increases the expression of proinflammatory chemokines in human pancreatic islets. *Diabetologia* (2014) 57(3):502–11. doi:10.1007/s00125-013-3135-2
46. Ferraro A, Socci C, Stabilini A, Valle A, Monti P, Piemonti L, et al. Expansion of Th17 cells and functional defects in T regulatory cells are key features of the pancreatic lymph nodes in patients with type 1 diabetes. *Diabetes* (2011) 60(11):2903–13. doi:10.2337/db11-0090
47. Nurieva R, Yang XO, Martinez G, Zhang Y, Panopoulos AD, Ma L, et al. Essential autocrine regulation by IL-21 in the generation of inflammatory T cells. *Nature* (2007) 448:480–3. doi:10.1038/nature05969
48. Korn T, Bettelli E, Gao W, Awasthi A, Jäger A, Strom TB, et al. IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. *Nature* (2007) 448:484–7. doi:10.1038/nature05970
49. Spolski R, Kashyap M, Robinson C, Yu Z, Leonard WJ. IL-21 signaling is critical for the development of type I diabetes in the NOD mouse. *Proc Natl Acad Sci U S A* (2008) 105:14028–33. doi:10.1073/pnas.0804358105
50. Sutherland AP, Van Belle T, Wurster AL, Suto A, Michaud M, Zhang D, et al. Interleukin-21 is required for the development of type 1 diabetes in NOD mice. *Diabetes* (2009) 58:1144–55. doi:10.2337/db08-0882
51. Rydén AK, Perdue NR, Pagni PP, Gibson CB, Ratliff SS, Kirk RK, et al. Anti-IL-21 monoclonal antibody combined with liraglutide effectively reverses established hyperglycemia in mouse models of type 1 diabetes. *J Autoimmun* (2017) 84:65–74. doi:10.1016/j.jaut.2017.07.006

52. Parrish-Novak J, Dillon SR, Nelson A, Hammond A, Sprecher C, Gross JA, et al. Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function. *Nature* (2000) 408:57–63. doi:10.1038/35040504
53. Spolski R, Leonard WJ. Interleukin-21: basic biology and implications for cancer and autoimmunity. *Annu Rev Immunol* (2008) 26:57–79. doi:10.1146/annurev.immunol.26.021607.090316
54. Van Belle TL, Nierkens S, Arens R, von Herrath MG. Interleukin-21 receptor-mediated signals control autoreactive T cell infiltration in pancreatic islets. *Immunity* (2012) 36:1060–72. doi:10.1016/j.immuni.2012.04.005
55. Vinuesa CG, Tangye SG, Moser B, Mackay CR. Follicular B helper T cells in antibody responses and autoimmunity. *Nat Rev Immunol* (2005) 5:853–65. doi:10.1038/nri1714
56. Regnell SE, Lernmark Å. Early prediction of autoimmune (type 1) diabetes. *Diabetologia* (2017) 60(8):1370–81. doi:10.1007/s00125-017-4308-1
57. Kenefeck R, Wang CJ, Kapadi T, Wardzinski L, Attridge K, Clough LE, et al. Follicular helper T cell signature in type 1 diabetes. *J Clin Invest* (2015) 125:292–303. doi:10.1172/JCI76238
58. Xu X, Shi Y, Cai Y, Zhang Q, Yang F, Chen H, et al. Inhibition of increased circulating Tfh cell by anti-CD20 monoclonal antibody in patients with type 1 diabetes. *PLoS One* (2013) 8:e79858. doi:10.1371/journal.pone.0079858
59. Ferreira RC, Simons HZ, Thompson WS, Cutler AJ, Dopico XC, Smyth DJ, et al. IL-21 production by CD4 effector T cells and frequency of circulating follicular helper T cells are increased in type 1 diabetes patients. *Diabetologia* (2015) 58:781–90. doi:10.1007/s00125-015-3509-8
60. Viisanen T, Ihantola EL, Näntö-Salonen K, Hyöty H, Nurminen N, Selvenius J, et al. Circulating CXCR5+PD-1+ICOS+ follicular T helper cells are increased close to the diagnosis of type 1 diabetes in children with multiple autoantibodies. *Diabetes* (2017) 66(2):437–47. doi:10.2337/db16-0714
61. Takahashi T, Kuniyasu Y, Toda M, Sakaguchi N, Itoh M, Iwata M, et al. Immunologic self-tolerance maintained by CD25+CD4+ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. *Int Immunol* (1998) 10(12):1969–80. doi:10.1093/intimm/10.12.1969
62. Boyman O, Sprent J. The role of interleukin-2 during homeostasis and activation of the immune system. *Nat Rev Immunol* (2012) 12(3):180–90. doi:10.1038/nri3156
63. Todd JA, Aitman TJ, Cornall RJ, Ghosh S, Hall JR, Hearne CM, et al. Genetic analysis of autoimmune type 1 diabetes mellitus in mice. *Nature* (1991) 351:542–7. doi:10.1038/351542a0
64. Yamanouchi J, Rainbow D, Serra P, Howlett S, Hunter K, Garner VE, et al. Interleukin-2 gene variation impairs regulatory T cell function and causes autoimmunity. *Nat Genet* (2007) 39:329–37. doi:10.1038/ng1958
65. Vella A, Cooper JD, Lowe CE, Walker N, Nutland S, Widmer B, et al. Localization of a type 1 diabetes locus in the IL2RA/CD25 region by use of tag single-nucleotide polymorphisms. *Am J Hum Genet* (2005) 76:773–9. doi:10.1086/429843
66. Lowe CE, Cooper JD, Brusko T, Walker NM, Smyth DJ, Bailey R, et al. Large-scale genetic fine mapping and genotype-phenotype associations implicate polymorphism in the IL2RA region in type 1 diabetes. *Nat Genet* (2007) 39:1074–82. doi:10.1038/ng2102
67. Todd JA, Walker NM, Cooper JD, Smyth DJ, Downes K, Plagnol V, et al. Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes. *Nat Genet* (2007) 39:857–64. doi:10.1038/ng2068
68. Dendrou CA, Plagnol V, Fung E, Yang JH, Downes K, Cooper JD, et al. Cell-specific protein phenotypes for the autoimmune locus IL2RA using a genotype-selectable human bioresource. *Nat Genet* (2009) 41:1011–5. doi:10.1038/ng.434
69. Schwedhelm K, Thorpe J, Murray SA, Gavin M, Speake C, Greenbaum C, et al. Attenuated IL-2R signaling in CD4 memory T cells of T1D subjects is intrinsic and dependent on activation state. *Clin Immunol* (2017) 181:67–74. doi:10.1016/j.clim.2017.06.004
70. Todd JA, Evangelou M, Cutler AJ, Pekalski ML, Walker NM, Stevens HE, et al. Regulatory T cell responses in participants with type 1 diabetes after a single dose of interleukin-2: a non-randomised, open label, adaptive dose-finding trial. *PLoS Med* (2016) 13(10):e1002139. doi:10.1371/journal.pmed.1002139
71. Lu J, Zhang C, Li L, Xue W, Zhang C, Zhang X. Unique features of pancreatic-resident regulatory T cells in autoimmune type 1 diabetes. *Front Immunol* (2017) 8:1235. doi:10.3389/fimmu.2017.01235
72. Burzyn D, Benoist C, Mathis D. Regulatory T cells in nonlymphoid tissues. *Nat Immunol* (2013) 14(10):1007–13. doi:10.1038/ni.2683
73. Herman AE, Freeman GJ, Mathis D, Benoist C. CD4+CD25+ T regulatory cells dependent on ICOS promote regulation of effector cells in the prediabetic lesion. *J Exp Med* (2004) 199(11):1479–89. doi:10.1084/jem.20040179
74. Kornete M, Sgouroudis E, Piccirillo CA. ICOS-dependent homeostasis and function of Foxp3+ regulatory T cells in islets of nonobese diabetic mice. *J Immunol* (2012) 188(3):1064–74. doi:10.4049/jimmunol.1101303
75. Tan TG, Mathis D, Benoist C. Singular role for T-BET+CXCR3+ regulatory T cells in protection from autoimmune diabetes. *Proc Natl Acad Sci U S A* (2016) 113(49):14103–8. doi:10.1073/pnas.1616710113
76. Green EA, Gorelik L, McGregor CM, Tran EH, Flavell RA. CD4+CD25+ T regulatory cells control anti-islet CD8+ T cells through TGF-beta-TGF-beta receptor interactions in type 1 diabetes. *Proc Natl Acad Sci U S A* (2003) 100(19):10878–83. doi:10.1073/pnas.1834400100
77. Muoio DM, Newgard CB. Mechanisms of disease: molecular and metabolic mechanisms of insulin resistance and beta-cell failure in type 2 diabetes. *Nat Rev Mol Cell Biol* (2008) 9(3):193–205. doi:10.1038/nrm2327
78. Lombardi A, Tomer Y. Interferon alpha impairs insulin production in human beta cells via endoplasmic reticulum stress. *J Autoimmun* (2017) 80:48–55. doi:10.1016/j.jaut.2017.02.002
79. Hansen JB, Tonnesen MF, Madsen AN, Hagedorn PH, Friberg J, Grunnet LG, et al. Divalent metal transporter 1 regulates iron-mediated ROS and pancreatic β cell fate in response to cytokines. *Cell Metab* (2012) 16(4):449–61. doi:10.1016/j.cmet.2012.09.001
80. Horwitz MS, Bradley LM, Harbertson J, Krahl T, Lee J, Sarvetnick N. Diabetes induced by Coxsackie virus: initiation by bystander damage and not molecular mimicry. *Nat Med* (1998) 4(7):781–5. doi:10.1038/nm0798-781
81. Tersey SA, Nishiki Y, Templin AT, Cabrera SM, Stull ND, Colvin SC, et al. Islet β-cell endoplasmic reticulum stress precedes the onset of type 1 diabetes in the nonobese diabetic mouse model. *Diabetes* (2012) 61(4):818–27. doi:10.2337/db11-1293
82. Engin F, Yermalovich A, Nguyen T, Hummasti S, Fu W, Eizirik DL, et al. Restoration of the unfolded protein response in pancreatic β cells protects mice against type 1 diabetes. *Sci Transl Med* (2013) 5(211):211ra156. doi:10.1126/scitranslmed.3006534
83. Meyerovich K, Ortis F, Allagnat F, Cardozo AK. Endoplasmic reticulum stress and the unfolded protein response in pancreatic islet inflammation. *J Mol Endocrinol* (2016) 57(1):R1–17. doi:10.1530/JME-15-0306
84. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* (1993) 259(5091):87–91. doi:10.1126/science.7678183
85. Pickup JC, Mattock MB, Chusney GD, Burt D. NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. *Diabetologia* (1997) 40:1286–92. doi:10.1007/s001250050822
86. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* (2001) 286:327–34. doi:10.1001/jama.286.3.327
87. Spranger J, Kroke A, Möhlig M, Hoffmann K, Bergmann MM, Ristow M, et al. Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European prospective investigation into cancer and nutrition (EPIC)-potsdam study. *Diabetes* (2003) 52:812–7. doi:10.2337/diabetes.52.3.812
88. Herder C, Brunner EJ, Rathmann W, Strassburger K, Tabák AG, Schloot NC, et al. Elevated levels of the anti-inflammatory interleukin-1 receptor antagonist precede the onset of type 2 diabetes: the Whitehall II study. *Diabetes Care* (2009) 32:421–3. doi:10.2337/dc08-1161
89. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. *Nat Rev Immunol* (2011) 11:98–107. doi:10.1038/nri2925
90. Nikolajczyk BS, Jagannathan-Bogdan M, Shin H, Gyurko R. State of the union between metabolism and the immune system in type 2 diabetes. *Genes Immun* (2011) 12:239–50. doi:10.1038/gene.2011.14
91. Nguyen MT, Favelyukis S, Nguyen AK, Reichart D, Scott PA, Jenn A, et al. A subpopulation of macrophages infiltrates hypertrophic adipose tissue

and is activated by free fatty acids via toll-like receptors 2 and 4 and JNK-dependent pathways. *J Biol Chem* (2007) 282:35279–92. doi:10.1074/jbc.M706762200
92. Ahmad R, Al-Mass A, Atizado V, Al-Hubail A, Al-Ghimlas F, Al-Arouj M, et al. Elevated expression of the toll-like receptors 2 and 4 in obese individuals: its significance for obesity-induced inflammation. *J Inflamm (Lond)* (2012) 9(1):48. doi:10.1186/1476-9255-9-48
93. Ehses J, Meier D, Wueest S, Rytka J, Boller S, Wielinga P, et al. Toll-like receptor 2-deficient mice are protected from insulin resistance and beta cell dysfunction induced by a high-fat diet. *Diabetologia* (2010) 53:1795–806. doi:10.1007/s00125-010-1747-3
94. Caricilli AM, Nascimento PH, Pauli JR, Tsukumo DM, Velloso LA, Carvalheira JB, et al. Inhibition of toll-like receptor 2 expression improves insulin sensitivity and signaling in muscle and white adipose tissue of mice fed a high-fat diet. *J Endocrinol* (2008) 199:399–406. doi:10.1677/JOE-08-0354
95. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* (2003) 112(12):1796–808. doi:10.1172/JCI200319246
96. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* (2003) 112(12):1821–30. doi:10.1172/JCI200319451
97. Xia C, Rao X, Zhong J. Role of T lymphocytes in type 2 diabetes and diabetes-associated inflammation. *J Diabetes Res* (2017) 2017:6494795. doi:10.1155/2017/6494795
98. Maedler K, Sergeev P, Ris F, Oberholzer J, Joller-Jemelka HI, Spinas GA, et al. Glucose-induced β-cell production of interleukin-1β contributes to glucotoxicity in human pancreatic islets. *J Clin Invest* (2002) 110:851–60. doi:10.1172/JCI200215318
99. Ehses JA, Perren A, Eppler E, Ribaux P, Pospisilik JA, Maor-Cahn R, et al. Increased number of islet-associated macrophages in type 2 diabetes. *Diabetes* (2007) 56:2356–70. doi:10.2337/db06-1650
100. Nackiewicz D, Dan M, He W, Kim R, Salmi A, Rütti S, et al. TLR2/6 and TLR4-activated macrophages contribute to islet inflammation and impair beta cell insulin gene expression via IL-1 and IL-6. *Diabetologia* (2014) 57(8):1645–54. doi:10.1007/s00125-014-3249-1
101. Yin J, Peng Y, Wu J, Wang Y, Yao L. Toll-like receptor 2/4 links to free fatty acid-induced inflammation and β-cell dysfunction. *J Leukoc Biol* (2014) 95(1):47–52. doi:10.1189/jlb.0313143
102. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest* (2007) 117:175–84. doi:10.1172/JCI29881
103. Boutens L, Stienstra R. Adipose tissue macrophages: going off track during obesity. *Diabetologia* (2016) 59(5):879–94. doi:10.1007/s00125-016-3904-9
104. Wu H, Ghosh S, Perrard XD, Feng L, Garcia GE, Perrard JL, et al. T-cell accumulation and regulated on activation, normal T cell expressed and secreted upregulation in adipose tissue in obesity. *Circulation* (2007) 115:1029–38. doi:10.1161/CIRCULATIONAHA.106.638379
105. Kintscher U, Hartge M, Hess K, Foryst-Ludwig A, Clemenz M, Wabitsch M, et al. T-lymphocyte infiltration in visceral adipose tissue: a primary event in adipose tissue inflammation and the development of obesity-mediated insulin resistance. *Arterioscler Thromb Vasc Biol* (2008) 28(7):1304–10. doi:10.1161/ATVBAHA.108.165100
106. Nishimura S, Manabe I, Nagasaki M, Eto K, Yamashita H, Ohsugi M, et al. CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nat Med* (2009) 15(8):914–20. doi:10.1038/nm.1964
107. Rausch ME, Weisberg S, Vardhana P, Tortoriello DV. Obesity in C57BL/6J mice is characterized by adipose tissue hypoxia and cytotoxic T-cell infiltration. *Int J Obes (Lond)* (2008) 32(3):451–63. doi:10.1038/sj.ijo.0803744
108. Rocha VZ, Folco EJ, Sukhova G, Shimizu K, Gotsman I, Vernon AH, et al. Interferon-gamma, a Th1 cytokine, regulates fat inflammation: a role for adaptive immunity in obesity. *Circ Res* (2008) 103(5):467–76. doi:10.1161/CIRCRESAHA.108.177105
109. McLaughlin T, Liu LF, Lamendola C, Shen L, Morton J, Rivas H, et al. T-cell profile in adipose tissue is associated with insulin resistance and systemic inflammation in humans. *Arterioscler Thromb Vasc Biol* (2014) 34(12):2637–43. doi:10.1161/ATVBAHA.114.304636
110. Shirakawa K, Yan X, Shinmura K, Endo J, Kataoka M, Katsumata Y, et al. Obesity accelerates T cell senescence in murine visceral adipose tissue. *J Clin Invest* (2016) 126(12):4626–39. doi:10.1172/JCI88606
111. Lynch L, Nowak M, Varghese B, Clark J, Hogan AE, Toxavidis V, et al. Adipose tissue invariant NKT cells protect against diet-induced obesity and metabolic disorder through regulatory cytokine production. *Immunity* (2012) 37(3):574–87. doi:10.1016/j.immuni.2012.06.016
112. Lynch L, O'Shea D, Winter DC, Geoghegan J, Doherty DG, O'Farrelly C. Invariant NKT cells and CD1d(+) cells amass in human omentum and are depleted in patients with cancer and obesity. *Eur J Immunol* (2009) 39:1893–901. doi:10.1002/eji.200939349
113. Bendelac A, Savage PB, Teyton L. The biology of NKT cells. *Annu Rev Immunol* (2007) 25:297–336. doi:10.1146/annurev.immunol.25.022106.141711
114. Godfrey DI, Berzins SP. Control points in NKT-cell development. *Nat Rev Immunol* (2007) 7(7):505–18. doi:10.1038/nri2116
115. Huh JY, Park J, Kim JI, Park YJ, Lee YK, Kim JB. Deletion of CD1d in adipocytes aggravates adipose tissue inflammation and insulin resistance in obesity. *Diabetes* (2017) 66(4):835–47. doi:10.2337/db16-1122
116. Chehimi M, Vidal H, Eljaafari A. Pathogenic role of IL-17-producing immune cells in obesity, and related inflammatory diseases. *J Clin Med* (2017) 6(7):E68. doi:10.3390/jcm6070068
117. Zeng C, Shi X, Zhang B, Liu H, Zhang L, Ding W, et al. The imbalance of Th17/Th1/Tregs in patients with type 2 diabetes: relationship with metabolic factors and complications. *J Mol Med (Berl)* (2012) 90(2):175–86. doi:10.1007/s00109-011-0816-5
118. Jagannathan-Bogdan M, McDonnell ME, Shin H, Rehman Q, Hasturk H, Apovian CM, et al. Elevated proinflammatory cytokine production by a skewed T cell compartment requires monocytes and promotes inflammation in type 2 diabetes. *J Immunol* (2011) 186(2):1162–72. doi:10.4049/jimmunol.1002615
119. Winer S, Paltser G, Chan Y, Tsui H, Engleman E, Winer D, et al. Obesity predisposes to Th17 bias. *Eur J Immunol* (2009) 39(9):2629–35. doi:10.1002/eji.200838893
120. Bertola A, Ciucci T, Rousseau D, Bourlier V, Duffaut C, Bonnafous S, et al. Identification of adipose tissue dendritic cells correlated with obesity-associated insulin-resistance and inducing Th17 responses in mice and patients. *Diabetes* (2012) 61(9):2238–47. doi:10.2337/db11-1274
121. Zúñiga LA, Shen WJ, Joyce-Shaikh B, Pyatnova EA, Richards AG, Thom C, et al. IL-17 regulates adipogenesis, glucose homeostasis, and obesity. *J Immunol* (2010) 185(11):6947–59. doi:10.4049/jimmunol.1001269
122. Kolodin D, van Panhuys N, Li C, Magnuson AM, Cipolletta D, Miller CM, et al. Antigen- and cytokine-driven accumulation of regulatory T cells in visceral adipose tissue of lean mice. *Cell Metab* (2015) 21(4):543–57. doi:10.1016/j.cmet.2015.03.005
123. Bapat SP, Myoung Suh J, Fang S, Liu S, Zhang Y, Cheng A, et al. Depletion of fat-resident Treg cells prevents age-associated insulin resistance. *Nature* (2015) 528(7580):137–41. doi:10.1038/nature16151
124. Schmitz J, Evers N, Awazawa M, Nicholls HT, Brönneke HS, Dietrich A, et al. Obesogenic memory can confer long-term increases in adipose tissue but not liver inflammation and insulin resistance after weight loss. *Mol Metab* (2016) 5(5):328–39. doi:10.1016/j.molmet.2015.12.001
125. Shirakawa K, Endo J, Katsumata Y, Yamamoto T, Kataoka M, Isobe S, et al. Negative legacy of obesity. *PLoS One* (2017) 12(10):e0186303. doi:10.1371/journal.pone.0186303
126. Yanagisawa K, Yue S, van der Vliet HJ, Wang R, Alatrakchi N, Golden-Mason L, et al. Ex vivo analysis of resident hepatic pro-inflammatory CD1d-reactive T cells and hepatocyte surface CD1d expression in hepatitis C. *J Viral Hepat* (2013) 20(8):556–65. doi:10.1111/jvh.12081
127. Tilloy F, Treiner E, Park SH, Garcia C, Lemonnier F, de la Salle H, et al. An invariant T cell receptor alpha chain defines a novel TAP-independent major histocompatibility complex class Ib-restricted alpha/beta T cell subpopulation in mammals. *J Exp Med* (1999) 189(12):1907–21. doi:10.1084/jem.189.12.1907
128. Magalhaes I, Pingris K, Poitou C, Bessoles S, Venteclef N, Kiaf B, et al. Mucosal-associated invariant T cell alterations in obese and type 2 diabetic patients. *J Clin Invest* (2015) 125(4):1752–62. doi:10.1172/JCI78941
129. Rouxel O, Da Silva J, Beaudoin L, Nel I, Tard C, Cagninacci L, et al. Cytotoxic and regulatory roles of mucosal-associated invariant T cells in type 1 diabetes. *Nat Immunol* (2017) 18(12):1321–31. doi:10.1038/ni.3854

130. Hiejima E, Kawai T, Nakase H, Tsuruyama T, Morimoto T, Yasumi T, et al. Reduced numbers and proapoptotic features of mucosal-associated invariant T cells as a characteristic finding in patients with inflammatory bowel disease. *Inflamm Bowel Dis* (2015) 21(7):1529–40. doi:10.1097/MIB.0000000000000397
131. Wen L, Ley RE, Volchkov PY, Stranges PB, Avanesyan L, Stonebraker AC, et al. Innate immunity and intestinal microbiota in the development of Type 1 diabetes. *Nature* (2008) 455(7216):1109–13. doi:10.1038/nature07336
132. Kostic AD, Gevers D, Siljander H, Vatanen T, Hyötyläinen T, Hämäläinen AM, et al. The dynamics of the human infant gut microbiome in development and in progression toward type 1 diabetes. *Cell Host Microbe* (2015) 17(2):260–73. doi:10.1016/j.chom.2015.01.001
133. Yu H, Gagliani N, Ishigame H, Huber S, Zhu S, Esplugues E, et al. Intestinal type 1 regulatory T cells migrate to periphery to suppress diabetogenic T cells and prevent diabetes development. *Proc Natl Acad Sci U S A* (2017) 114(39):10443–8. doi:10.1073/pnas.1705599114
134. Mariño E, Richards JL, McLeod KH, Stanley D, Yap YA, Knight J, et al. Gut microbial metabolites limit the frequency of autoimmune T cells and protect against type 1 diabetes. *Nat Immunol* (2017) 18(5):552–62. doi:10.1038/ni.3713
135. DeFronzo RA, Tripathy D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care* (2009) 32(Suppl 2):S157–63. doi:10.2337/dc09-S302
136. Osborn O, Olefsky JM. The cellular and signaling networks linking the immune system and metabolism in disease. *Nat Med* (2012) 18(3):363–74. doi:10.1038/nm.2627
137. Fink LN, Costford SR, Lee YS, Jensen TE, Bilan PJ, Oberbach A, et al. Pro-inflammatory macrophages increase in skeletal muscle of high fat-fed mice and correlate with metabolic risk markers in humans. *Obesity (Silver Spring)* (2014) 22(3):747–57. doi:10.1002/oby.20615
138. Hong EG, Ko HJ, Cho YR, Kim HJ, Ma Z, Yu TY, et al. Interleukin-10 prevents diet-induced insulin resistance by attenuating macrophage and cytokine response in skeletal muscle. *Diabetes* (2009) 58(11):2525–35. doi:10.2337/db08-1261
139. Khan IM, Dai Perrard XY, Perrard JL, Mansoori A, Smith CW, Wu H, et al. Attenuated adipose tissue and skeletal muscle inflammation in obese mice with combined CD4+ and CD8+ T cell deficiency. *Atherosclerosis* (2014) 233(2):419–28. doi:10.1016/j.atherosclerosis.2014.01.011
140. Jiang E, Perrard XD, Yang D, Khan IM, Perrard JL, Smith CW, et al. Essential role of CD11a in $CD8^+$ T-cell accumulation and activation in adipose tissue. *Arterioscler Thromb Vasc Biol* (2013) 34:34–43. doi:10.1161/ATVBAHA.113.302077
141. Zhao C, Wang Z, Robertson MW, Davies JD. Cachexia in the non-obese diabetic mouse is associated with CD4+ T-cell lymphopenia. *Immunology* (2008) 125(1):48–58. doi:10.1111/j.1365-2567.2008.02819.x
142. Stentz FB, Kitabchi AE. Transcriptome and proteome expressions involved in insulin resistance in muscle and activated T-lymphocytes of patients with type 2 diabetes. *Genomics Proteomics Bioinformatics* (2007) 5(3–4):216–35. doi:10.1016/S1672-0229(08)60009-1
143. Schiaffino S, Pereira MG, Ciciliot S, Rovere-Querini P. Regulatory T cells and skeletal muscle regeneration. *FEBS J* (2017) 284(4):517–24. doi:10.1111/febs.13827
144. Klein L, Kyewski B, Allen PM, Hogquist KA. Positive and negative selection of the T cell repertoire: what thymocytes see (and don't see). *Nat Rev Immunol* (2014) 14(6):377–91. doi:10.1038/nri3667
145. Bergan-Roller HE, Sheridan MA. The growth hormone signaling system: insights into coordinating the anabolic and catabolic actions of growth hormone. *Gen Comp Endocrinol* (2017) 258:119–33. doi:10.1016/j.ygcen.2017.07.028
146. Weigent DA. Lymphocyte GH-axis hormones in immunity. *Cell Immunol* (2013) 285(1–2):118–32. doi:10.1016/j.cellimm.2013.10.003
147. Davoodi-Semiromi A, Laloraya M, Kumar GP, Purohit S, Jha RK, She JX. A mutant Stat5b with weaker DNA binding affinity defines a key defective pathway in nonobese diabetic mice. *J Biol Chem* (2004) 279(12):11553–61. doi:10.1074/jbc.M312110200
148. Murawski MR, Litherland SA, Clare-Salzler MJ, Davoodi-Semiromi A. Upregulation of Foxp3 expression in mouse and human Treg is IL-2/STAT5 dependent: implications for the NOD STAT5B mutation in diabetes pathogenesis. *Ann N Y Acad Sci* (2006) 1079:198–204. doi:10.1196/annals.1375.031
149. Villares R, Kakabadse D, Juarranz Y, Gomariz RP, Martínez-A C, Mellado M. Growth hormone prevents the development of autoimmune diabetes. *Proc Natl Acad Sci U S A* (2013) 110(48):E4619–27. doi:10.1073/pnas.1314985110
150. Blethen SL, Allen DB, Graves D, August G, Moshang T, Rosenfeld R. Safety of recombinant deoxyribonucleic acid-derived growth hormone: the national cooperative growth study experience. *J Clin Endocrinol Metab* (1996) 81:1704–10. doi:10.1210/jcem.81.5.8626820
151. Cutfield WS, Wilton P, Bennmarker H, Albertsson-Wikland K, Chatelain P, Ranke MB, et al. Incidence of diabetes mellitus and impaired glucose tolerance in children and adolescents receiving growth-hormone treatment. *Lancet* (2000) 355:610–3. doi:10.1016/S0140-6736(99)04055-6
152. Child CJ, Zimmermann AG, Scott RS, Cutler GB Jr, Battelino T, Blum WF, et al. Prevalence and incidence of diabetes mellitus in GH-treated children and adolescents: analysis from the GeNeSIS observational research program. *J Clin Endocrinol Metab* (2011) 96:E1025–34. doi:10.1210/jc.2010-3023
153. Møller N, Jørgensen JO. Effects of growth hormone on glucose, lipid, and protein metabolism in human subjects. *Endocr Rev* (2009) 30:152–77. doi:10.1210/er.2008-0027
154. Bonfig W, Molz K, Woelfle J, Hofer SE, Hauffa BP, Schoenau E, et al. Metabolic safety of growth hormone in type 1 diabetes and idiopathic growth hormone deficiency. *J Pediatr* (2013) 163(4):1095–8.e4. doi:10.1016/j.jpeds.2013.04.045
155. Kim SH, Park MJ. Effects of growth hormone on glucose metabolism and insulin resistance in human. *Ann Pediatr Endocrinol Metab* (2017) 22(3):145–52. doi:10.6065/apem.2017.22.3.145
156. de Mello Coelho V, Villa-Verde DM, Farias-de-Oliveira DA, de Brito JM, Dardenne M, Savino W. Functional insulin-like growth factor-1/insulin-like growth factor-1 receptor-mediated circuit in human and murine thymic epithelial cells. *Neuroendocrinology* (2002) 75(2):139–50. doi:10.1159/000048230
157. Bergerot I, Fabien N, Maguer V, Thivolet C. Insulin-like growth factor-1 (IGF-1) protects NOD mice from insulitis and diabetes. *Clin Exp Immunol* (1995) 102:335–40. doi:10.1111/j.1365-2249.1995.tb03786.x
158. Kaino Y, Hirai H, Ito T, Kida K. Insulin-like growth factor I (IGF-I) delays the onset of diabetes in non-obese diabetic (NOD) mice. *Diabetes Res Clin Pract* (1996) 34:7–11. doi:10.1016/S0168-8227(96)01326-5
159. Chen W, Salojin KV, Mi QS, Grattan M, Meagher TC, Zucker P, et al. Insulin-like growth factor (IGF)-I/IGF-binding protein-3 complex: therapeutic efficacy and mechanism of protection against type 1 diabetes. *Endocrinology* (2004) 145(2):627–38. doi:10.1210/en.2003-1274
160. Anguela XM, Tafuro S, Roca C, Callejas D, Agudo J, Obach M, et al. Nonviral-mediated hepatic expression of IGF-I increases Treg levels and suppresses autoimmune diabetes in mice. *Diabetes* (2013) 62(2):551–60. doi:10.2337/db11-1776
161. Bilbao D, Luciani L, Johannesson B, Piszczek A, Rosenthal N. Insulin-like growth factor-1 stimulates regulatory T cells and suppresses autoimmune disease. *EMBO Mol Med* (2014) 6(11):1423–35. doi:10.15252/emmm.201303376
162. George M, Ayuso E, Casellas A, Costa C, Devedjian JC, Bosch F. Beta cell expression of IGF-I leads to recovery from type 1 diabetes. *J Clin Invest* (2002) 109:1153–63. doi:10.1172/JCI0212969
163. Casellas A, Salavert A, Agudo J, Ayuso E, Jimenez V, Moya M, et al. Expression of IGF-I in pancreatic islets prevents lymphocytic infiltration and protects mice from type 1 diabetes. *Diabetes* (2006) 55:3246–55. doi:10.2337/db06-0328
164. Agudo J, Ayuso E, Jimenez V, Salavert A, Casellas A, Tafuro S, et al. IGF-I mediates regeneration of endocrine pancreas by increasing beta cell replication through cell cycle protein modulation in mice. *Diabetologia* (2008) 51:1862–72. doi:10.1007/s00125-008-1087-8
165. Ashwell JD, Lu FW, Vacchio MS. Glucocorticoids in T cell development and function. *Annu Rev Immunol* (2000) 18:309–45. doi:10.1146/annurev.immunol.18.1.309
166. Mittelstadt PR, Monteiro JP, Ashwell JD. Thymocyte responsiveness to endogenous glucocorticoids is required for immunological fitness. *J Clin Invest* (2012) 122(7):2384–94. doi:10.1172/JCI63067
167. Coutinho AE, Chapman KE. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. *Mol Cell Endocrinol* (2011) 335(1):2–13. doi:10.1016/j.mce.2010.04.005
168. Londono P, Komura A, Hara N, Zipris D. Brief dexamethasone treatment during acute infection prevents virus-induced autoimmune diabetes. *Clin Immunol* (2010) 135(3):401–11. doi:10.1016/j.clim.2010.01.007

169. Schäcke H, Döcke WD, Asadullah K. Mechanisms involved in the side effects of glucocorticoids. *Pharmacol Ther* (2002) 96(1):23–43. doi:10.1016/S0163-7258(02)00297-8
170. Fauci AS, Dale DC. The effect of in vivo hydrocortisone on subpopulations of human lymphocytes. *J Clin Invest* (1974) 3:240. doi:10.1172/JCI107544
171. Almawi WY, Beyhum HN, Rahme AA, Rieder MJ. Regulation of cytokine and cytokine receptor expression by glucocorticoids. *J Leukoc Biol* (1996) 60:563. doi:10.1002/jlb.60.5.563
172. Wiegers GJ, Labeur MS, Stec IE, Klinkert WE, Holsboer F, Reul JM. Glucocorticoids accelerate anti-T cell receptor-induced T cell growth. *J Immunol* (1995) 155(4):1893–902.
173. Wiegers GJ, Stec IE, Klinkert WE, Reul JM. Glucocorticoids regulate TCR-induced elevation of CD4: functional implications. *J Immunol* (2000) 164(12):6213–20. doi:10.4049/jimmunol.164.12.6213
174. Ramirez F, McKnight AJ, Silva A, Mason D. Glucocorticoids induce the expression of CD8 alpha chains on concanavalin A-activated rat CD4+ T cells: induction is inhibited by rat recombinant interleukin 4. *J Exp Med* (1992) 176(6):1551–9. doi:10.1084/jem.176.6.1551
175. Schäcke H, Schottelius A, Döcke WD, Strehlke P, Jaroch S, Schmees N, et al. Dissociation of transactivation from transrepression by a selective glucocorticoid receptor agonist leads to separation of therapeutic effects from side effects. *Proc Natl Acad Sci U S A* (2004) 101(1):227–32. doi:10.1073/pnas.0300372101
176. Barcala Tabarrozzi AE, Andreone L, Deckers J, Castro CN, Gimeno ML, Ariolfo L, et al. GR-independent down-modulation on GM-CSF bone marrow-derived dendritic cells by the selective glucocorticoid receptor modulator compound A. *Sci Rep* (2016) 6:36646. doi:10.1038/srep36646
177. De Bosscher K, Vanden Berghe W, Beck IM, Van Molle W, Hennuyer N, Hapgood J, et al. A fully dissociated compound of plant origin for inflammatory gene repression. *Proc Natl Acad Sci U S A* (2005) 102:15827–32. doi:10.1073/pnas.0505554102
178. Liberman AC, Antunica-Noguerol M, Ferraz-de-Paula V, Palermo-Neto J, Castro CN, Druker J, et al. Compound A, a dissociated glucocorticoid receptor modulator, inhibits T-bet (Th1) and induces GATA-3 (Th2) activity in immune cells. *PLoS One* (2012) 7(4):e35155. doi:10.1371/journal.pone.0035155
179. WHO. *Improving Preterm Birth Outcomes. Execute Summary*. Geneva: WHO (2015).
180. Lindsay RS, Lindsay RM, Waddell BJ, Seckl JR. Prenatal glucocorticoid exposure leads to offspring hyperglycaemia in the rat: studies with the 11 beta-hydroxysteroid dehydrogenase inhibitor carbenoxolone. *Diabetologia* (1996) 39:1299–305. doi:10.1007/s001250050573
181. Nyirenda MJ, Lindsay RS, Kenyon CJ, Burchell A, Seckl JR. Glucocorticoid exposure in late gestation permanently programs rat hepatic phosphoenol-pyruvate carboxykinase and glucocorticoid receptor expression and causes glucose intolerance in adult offspring. *J Clin Invest* (1998) 101:2174–81. doi:10.1172/JCI1567
182. De Blasio MJ, Dodic M, Jefferies AJ, Moritz KM, Wintour EM, Owens JA. Maternal exposure to dexamethasone or cortisol in early pregnancy differentially alters insulin secretion and glucose homeostasis in adult male sheep offspring. *Am J Physiol Endocrinol Metab* (2007) 293(1):E75–82. doi:10.1152/ajpendo.00689.2006
183. Kelly BA, Lewandowski AJ, Worton SA, Davis EF, Lazdam M, Francis J, et al. Antenatal glucocorticoid exposure and long-term alterations in aortic function and glucose metabolism. *Pediatrics* (2012) 129(5):e1282–90. doi:10.1542/peds.2011-3175
184. Diepenbruck I, Much CC, Krumbholz A, Kolster M, Thieme R, Thieme D, et al. Effect of prenatal steroid treatment on the developing immune system. *J Mol Med (Berl)* (2013) 91(11):1293–302. doi:10.1007/s00109-013-1069-2
185. Gieras A, Gehbauer C, Perna-Barrull D, Engler JB, Diepenbruck I, Glau L, et al. Prenatal administration of betamethasone causes changes in the T cell receptor repertoire influencing development of autoimmunity. *Front Immunol* (2017) 8:1505. doi:10.3389/fimmu.2017.01505
186. Greene NH, Pedersen LH, Liu S, Olsen J. Prenatal prescription corticosteroids and offspring diabetes: a national cohort study. *Int J Epidemiol* (2013) 42(1):186–93. doi:10.1093/ije/dys228
187. Gesina E, Tronche F, Herrera P, Duchene B, Tales W, Czernichow P, et al. Dissecting the role of glucocorticoids on pancreas development. *Diabetes* (2004) 53(9):2322–9. doi:10.2337/diabetes.53.9.2322
188. Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, et al. A role for ghrelin in the central regulation of feeding. *Nature* (2001) 409(6817):194–8. doi:10.1038/35051587
189. Inui A. Ghrelin: an orexigenic and somatotrophic signal from the stomach. *Nat Rev Neurosci* (2001) 2(8):551–60. doi:10.1038/35086018
190. Dixit VD, Schaffer EM, Pyle RS, Collins GD, Sakthivel SK, Palaniappan R, et al. Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T cells. *J Clin Invest* (2004) 114(1):57–66. doi:10.1172/JCI200421134
191. Lee JH, Patel K, Tae HJ, Lustig A, Kim JW, Mattson MP, et al. Ghrelin augments murine T-cell proliferation by activation of the phosphatidylinositol-3-kinase, extracellular signal-regulated kinase and protein kinase C signaling pathways. *FEBS Lett* (2014) 588(24):4708–19. doi:10.1016/j.febslet.2014.10.044
192. Dixit VD, Yang H, Sun Y, Weeraratna AT, Youm YH, Smith RG, et al. Ghrelin promotes thymopoiesis during aging. *J Clin Invest* (2007) 117(10):2778–90. doi:10.1172/JCI30248
193. Dixit VD, Yang H, Cooper-Jenkins A, Giri BB, Patel K, Taub DD. Reduction of T cell-derived ghrelin enhances proinflammatory cytokine expression: implications for age-associated increases in inflammation. *Blood* (2009) 113(21):5202–5. doi:10.1182/blood-2008-09-181255
194. Baena-Nieto G, Lomas-Romero IM, Mateos RM, Leal-Cosme N, Perez-Arana G, Aguilar-Diosdado M, et al. Ghrelin mitigates β-cell mass loss during insulitis in an animal model of autoimmune diabetes mellitus, the BioBreeding/Worcester rat. *Diabetes Metab Res Rev* (2017) 33(1):1–13. doi:10.1002/dmrr.2813
195. Orlova EG, Shirshev SV, Loginova OA. Leptin and ghrelin regulate dendritic cell maturation and dendritic cell induction of regulatory T-cells. *Dokl Biol Sci* (2015) 462:171–4. doi:10.1134/S001249661503014X
196. Friedman JM. Leptin, leptin receptors, and the control of body weight. *Nutr Rev* (1998) 56(2 Pt 2):s38–46; discussion s54–75. doi:10.1111/j.1753-4887.1998.tb01685.x
197. Gainsford T, Willson TA, Metcalf D, Handman E, McFarlane C, Ng A, et al. Leptin can induce proliferation, differentiation, and functional activation of hemopoietic cells. *Proc Natl Acad Sci U S A* (1996) 93(25):14564–8. doi:10.1073/pnas.93.25.14564
198. Bouloumié A, Drexler HC, Lafontan M, Busse R. Leptin, the product of Ob gene, promotes angiogenesis. *Circ Res* (1998) 83(10):1059–66. doi:10.1161/01.RES.83.10.1059
199. Chehab FF, Lim ME, Lu R. Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. *Nat Genet* (1996) 12(3):318–20. doi:10.1038/ng0396-318
200. Matarese G, Moschos S, Mantzoros CS. Leptin in immunology. *J Immunol* (2005) 174(6):3137–42. doi:10.4049/jimmunol.174.6.3137
201. Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature* (1998) 394(6696):897–901. doi:10.1038/29795
202. Lord GM, Matarese G, Howard JK, Bloom SR, Lechler RI. Leptin inhibits the anti-CD3-driven proliferation of peripheral blood T cells but enhances the production of proinflammatory cytokines. *J Leukoc Biol* (2002) 72(2):330–8.
203. Matarese G, Sanna V, Lechler RI, Sarvetnick N, Fontana S, Zappacosta S, et al. Leptin accelerates autoimmune diabetes in female NOD mice. *Diabetes* (2002) 51(5):1356–61. doi:10.2337/diabetes.51.5.1356
204. Lee CH, Reifsnyder PC, Naggert JK, Wasserfall C, Atkinson MA, Chen J, et al. Novel leptin receptor mutation in NOD/LtJ mice suppresses type 1 diabetes progression: I. Pathophysiological analysis. *Diabetes* (2005) 54(9):2525–32. doi:10.2337/diabetes.54.9.2525
205. Dandona P, Aljada A, Mohanty P, Ghanim H, Hamouda W, Assian E, et al. Insulin inhibits intranuclear nuclear factor kappaB and stimulates IkappaB in mononuclear cells in obese subjects: evidence for an anti-inflammatory effect? *J Clin Endocrinol Metab* (2001) 86(7):3257–65. doi:10.1210/jcem.86.7.7623
206. Viardot A, Grey ST, Mackay F, Chisholm D. Potential antiinflammatory role of insulin via the preferential polarization of effector T cells toward a T helper 2 phenotype. *Endocrinology* (2007) 148(1):346–53. doi:10.1210/en.2006-0686
207. Viardot A, Heilbronn LK, Samocha-Bonet D, Mackay F, Campbell LV, Samaras K. Obesity is associated with activated and insulin resistant immune cells. *Diabetes Metab Res Rev* (2012) 28(5):447–54. doi:10.1002/dmrr.2302

208. Otton R, Soriano FG, Verlengia R, Curi R. Diabetes induces apoptosis in lymphocytes. *J Endocrinol* (2004) 182(1):145–56. doi:10.1677/joe.0.1820145
209. Pedersen DJ, Guilherme A, Danai LV, Heyda L, Matevossian A, Cohen J, et al. A major role of insulin in promoting obesity-associated adipose tissue inflammation. *Mol Metab* (2015) 4(7):507–18. doi:10.1016/j.molmet.2015.04.003
210. Martinez-Sanchez ME, Hiriart M, Alvarez-Buylla ER. The CD4+ T cell regulatory network mediates inflammatory responses during acute hyperinsulinemia: a simulation study. *BMC Syst Biol* (2017) 11(1):64. doi:10.1186/s12918-017-0436-y
211. Han JM, Patterson SJ, Speck M, Ehses JA, Levings MK. Insulin inhibits IL-10-mediated regulatory T cell function: implications for obesity. *J Immunol* (2014) 192(2):623–9. doi:10.4049/jimmunol.1302181
212. Helderman JH, Strom TB. Specific insulin binding site on T and B lymphocytes as a marker of cell activation. *Nature* (1978) 274(5666):62–3. doi:10.1038/274062a0
213. Brown TJ, Ercolani L, Ginsberg BH. Properties and regulation of the T lymphocyte insulin receptor. *J Recept Res* (1983) 3:481–94. doi:10.3109/10799898309041854
214. Fischer HJ, Sie C, Schumann E, Witte AK, Dressel R, van den Brandt J, et al. The insulin receptor plays a critical role in T cell function and adaptive immunity. *J Immunol* (2017) 198(5):1910–20. doi:10.4049/jimmunol.1601011
215. Frauwirth KA, Riley JL, Harris MH, Parry RV, Rathmell JC, Plas DR, et al. The CD28 signaling pathway regulates glucose metabolism. *Immunity* (2002) 16(6):769–77. doi:10.1016/S1074-7613(02)00323-0
216. Jacobs SR, Herman CE, Maciver NJ, Wofford JA, Wieman HL, Hammen JJ, et al. Glucose uptake is limiting in T cell activation and requires CD28-mediated Akt-dependent and independent pathways. *J Immunol* (2008) 180(7):4476–86. doi:10.4049/jimmunol.180.7.4476
217. Jones N, Cronin JG, Dolton G, Panetti S, Schauenburg AJ, Galloway SAE, et al. Metabolic adaptation of human CD4+ and CD8+ T-cells to T-cell receptor-mediated stimulation. *Front Immunol* (2017) 8:1516. doi:10.3389/fimmu.2017.01516
218. Pellegrini I, Lebrun JJ, Ali S, Kelly PA. Expression of prolactin and its receptor in human lymphoid cells. *Mol Endocrinol* (1992) 6(7):1023–31. doi:10.1210/me.6.7.1023
219. Hawkins TA, Gala RR, Dunbar JC. The lymphocyte and macrophage profile in the pancreas and spleen of NOD mice: percentage of interleukin-2 and prolactin receptors on immunocompetent cell subsets. *J Reprod Immunol* (1996) 32(1):55–71. doi:10.1016/S0165-0378(96)00986-2
220. Spangelo BL, Hall NR, Ross PC, Goldstein AL. Stimulation of in vivo antibody production and concanavalin-A-induced mouse spleen cell mitogenesis by prolactin. *Immunopharmacology* (1987) 14(1):11–20. doi:10.1016/0162-3109(87)90004-X
221. Bernton EW, Meltzer MS, Holaday JW. Suppression of macrophage activation and T-lymphocyte function in hypoprolactinemic mice. *Science* (1988) 239(4838):401–4. doi:10.1126/science.3122324
222. Gala RR, Shevach EM. Influence of prolactin and growth hormone on the activation of dwarf mouse lymphocytes in vivo. *Proc Soc Exp Biol Med* (1993) 204(2):224–30. doi:10.3181/00379727-204-43657
223. Mooradian AD, Morley JE, Billington CJ, Slag MF, Elson MK, Shafer RB. Hyperprolactinaemia in male diabetics. *Postgrad Med J* (1985) 61(711):11–4. doi:10.1136/pgmj.61.711.11
224. Orbach H, Shoenfeld Y. Hyperprolactinemia and autoimmune diseases. *Autoimmun Rev* (2007) 6(8):537–42. doi:10.1016/j.autrev.2006.10.005
225. Arnold E, Rivera JC, Thebault S, Moreno-Páramo D, Quiroz-Mercado H, Quintanar-Stéphano A, et al. High levels of serum prolactin protect against diabetic retinopathy by increasing ocular vasoinhibins. *Diabetes* (2010) 59(12):3192–7. doi:10.2337/db10-0873
226. Daimon M, Kamba A, Murakami H, Mizushiri S, Osonoi S, Yamaichi M, et al. Association between serum prolactin levels and insulin resistance in non-diabetic men. *PLoS One* (2017) 12(4):e0175204. doi:10.1371/journal.pone.0175204
227. Ekinci EI, Torkamani N, Ramchand SK, Churilov L, Sikaris KA, Lu ZX, et al. Higher maternal serum prolactin levels are associated with reduced glucose tolerance during pregnancy. *J Diabetes Investig* (2017) 8(5):697–700. doi:10.1111/jdi.12634
228. Wang T, Lu J, Xu Y, Li M, Sun J, Zhang J, et al. Circulating prolactin associates with diabetes and impaired glucose regulation: a population-based study. *Diabetes Care* (2013) 36(7):1974–80. doi:10.2337/dc12-1893
229. Atwater I, Gondos B, DiBartolomeo R, Bazaes R, Jovanovic L. Pregnancy hormones prevent diabetes and reduce lymphocytic infiltration of islets in the NOD mouse. *Ann Clin Lab Sci* (2002) 32:87–92.
230. Holstad M, Sandler S. Prolactin protects against diabetes induced by multiple low doses of streptozotocin in mice. *J Endocrinol* (1999) 163(2):229–34. doi:10.1677/joe.0.1630229
231. Lau J, Börjesson A, Holstad M, Sandler S. Prolactin regulation of the expression of TNF-alpha, IFN-gamma and IL-10 by splenocytes in murine multiple low dose streptozotocin diabetes. *Immunol Lett* (2006) 102(1):25–30. doi:10.1016/j.imlet.2005.06.006
232. Hyslop CM, Tsai S, Shrivastava V, Santamaria P, Huang C. Prolactin as an adjunct for type 1 diabetes immunotherapy. *Endocrinology* (2016) 157(1):150–65. doi:10.1210/en.2015-1549
233. Hawkins TA, Gala RR, Dunbar JC. Prolactin modulates the incidence of diabetes in male and female NOD mice. *Autoimmunity* (1994) 18(3):155–62. doi:10.3109/08916939409007991
234. Tomio A, Schust DJ, Kawana K, Yasugi T, Kawana Y, Mahalingaiah S, et al. Prolactin can modulate CD4+ T-cell response through receptor-mediated alterations in the expression of T-bet. *Immunol Cell Biol* (2008) 86(7):616–21. doi:10.1038/icb.2008.29
235. Sorenson RL, Brelje TC, Roth C. Effects of steroid and lactogenic hormones on islets of Langerhans: a new hypothesis for the role of pregnancy steroids in the adaptation of islets to pregnancy. *Endocrinology* (1993) 133:2227–34. doi:10.1210/endo.133.5.8404674
236. Stout LE, Svensson AM, Sorenson RL. Prolactin regulation of islet-derived INS-1 cells: characteristics and immunocytochemical analysis of STAT5 translocation. *Endocrinology* (1997) 138(4):1592–603. doi:10.1210/endo.138.4.5089
237. Freemark M, Avril I, Fleenor D, Driscoll P, Petro A, Opara E, et al. Targeted deletion of the PRL receptor: effects on islet development, insulin production, and glucose tolerance. *Endocrinology* (2002) 143(4):1378–85. doi:10.1210/endo.143.4.8722
238. Huang C, Snider F, Cross JC. Prolactin receptor is required for normal glucose homoeostasis and modulation of beta-cell mass during pregnancy. *Endocrinology* (2009) 150:1618–26. doi:10.1210/en.2008-1003
239. Burbach JP, Luckman SM, Murphy D, Gainer H. Gene regulation in the magnocellular hypothalamo-neurohypophysial system. *Physiol Rev* (2001) 81(3):1197–267. doi:10.1152/physrev.2001.81.3.1197
240. Gimpl G, Fahrenholz F. The oxytocin receptor system: structure, function, and regulation. *Physiol Rev* (2001) 81(2):629–83. doi:10.1152/physrev.2001.81.2.629
241. Wang P, Yang HP, Tian S, Wang L, Wang SC, Zhang F, et al. Oxytocin-secreting system: a major part of the neuroendocrine center regulating immunologic activity. *J Neuroimmunol* (2015) 289:152–61. doi:10.1016/j.jneuroim.2015.11.001
242. Jin D, Liu HX, Hirai H, Torashima T, Nagai T, Lopatina O, et al. CD38 is critical for social behaviour by regulating oxytocin secretion. *Nature* (2007) 446(7131):41–5. doi:10.1038/nature05526
243. Chen J, Chen YG, Reifsnyder PC, Schott WH, Lee CH, Osborne M, et al. Targeted disruption of CD38 accelerates autoimmune diabetes in NOD/Lt mice by enhancing autoimmunity in an ADP ribosyltransferase 2-dependent fashion. *J Immunol* (2006) 176(8):4590–9. doi:10.4049/jimmunol.176.8.4590
244. Watanabe S, Wei FY, Matsunaga T, Matsunaga N, Kaitsuka T, Tomizawa K. Oxytocin protects against stress-induced cell death in murine pancreatic β-cells. *Sci Rep* (2016) 6:25185. doi:10.1038/srep25185
245. Takayanagi Y, Kasahara Y, Onaka T, Takahashi N, Kawada T, Nishimori K. Oxytocin receptor-deficient mice developed late-onset obesity. *Neuroreport* (2008) 19(9):951–5. doi:10.1097/WNR.0b013e3283021ca9
246. Camerino C. Low sympathetic tone and obese phenotype in oxytocin-deficient mice. *Obesity (Silver Spring)* (2009) 17(5):980–4. doi:10.1038/oby.2009.12
247. Maejima Y, Iwasaki Y, Yamahara Y, Kodaira M, Sedbazar U, Yada T. Peripheral oxytocin treatment ameliorates obesity by reducing food intake and visceral fat mass. *Aging (Albany NY)* (2011) 3(12):1169–77. doi:10.18632/aging.100408
248. Zhang H, Wu C, Chen Q, Chen X, Xu Z, Wu J, et al. Treatment of obesity and diabetes using oxytocin or analogs in patients and mouse models. *PLoS One* (2013) 8(5):e61477. doi:10.1371/journal.pone.0061477

249. Elabd SK, Sabry I, Mohasseb M, Algendy A. Oxytocin as a novel therapeutic option for type I diabetes and diabetic osteopathy. *Endocr Regul* (2014) 48(2):87–102. doi:10.4149/endo_2014_02_87
250. Altirriba J, Poher AL, Caillon A, Arsenijevic D, Veyrat-Durebex C, Lyautey J, et al. Divergent effects of oxytocin treatment of obese diabetic mice on adiposity and diabetes. *Endocrinology* (2014) 155(11):4189–201. doi:10.1210/en.2014-1466
251. Klement J, Ott V, Rapp K, Brede S, Piccinini F, Cobelli C, et al. Oxytocin improves β-cell responsivity and glucose tolerance in healthy men. *Diabetes* (2017) 66(2):264–71. doi:10.2337/db16-0569
252. Ngo ST, Steyn FJ, McCombe PA. Gender differences in autoimmune disease. *Front Neuroendocrinol* (2014) 35(3):347–69. doi:10.1016/j.yfrne.2014.04.004
253. Ortona E, Pierdominici M, Maselli A, Veroni C, Aloisi F, Shoenfeld Y. Sex-based differences in autoimmune diseases. *Ann Ist Super Sanita* (2016) 52(2):205–12. doi:10.4415/ANN_16_02_12
254. Kissick HT, Sanda MG, Dunn LK, Pellegrini KL, On ST, Noel JK, et al. Androgens alter T-cell immunity by inhibiting T-helper 1 differentiation. *Proc Natl Acad Sci U S A* (2014) 111(27):9887–92. doi:10.1073/pnas.1402468111
255. Trigunaite A, Dimo J, Jørgensen TN. Suppressive effects of androgens on the immune system. *Cell Immunol* (2015) 294(2):87–94. doi:10.1016/j.cellimm.2015.02.004
256. Li X, Wang B, Li Y, Wang L, Zhao X, Zhou X, et al. The Th1/Th2/Th17/Treg paradigm induced by stachydrine hydrochloride reduces uterine bleeding in RU486-induced abortion mice. *J Ethnopharmacol* (2013) 145(1):241–53. doi:10.1016/j.jep.2012.10.059
257. Tan IJ, Peeva E, Zandman-Goddard G. Hormonal modulation of the immune system – a spotlight on the role of progestogens. *Autoimmun Rev* (2015) 14(6):536–42. doi:10.1016/j.autrev.2015.02.004
258. Straub RH. The complex role of estrogens in inflammation. *Endocr Rev* (2007) 28(5):521–74. doi:10.1210/er.2007-0001
259. Prieto GA, Rosenstein Y. Oestradiol potentiates the suppressive function of human CD4 CD25 regulatory T cells by promoting their proliferation. *Immunology* (2006) 118(1):58–65. doi:10.1111/j.1365-2567.2006.02339.x
260. Gilmore W, Weiner LP, Correale J. Effect of estradiol on cytokine secretion by proteolipid protein-specific T cell clones isolated from multiple sclerosis patients and normal control subjects. *J Immunol* (1997) 158(1):446–51.
261. Kachapati K, Adams D, Bednar K, Ridgway WM. The non-obese diabetic (NOD) mouse as a model of human type 1 diabetes. *Methods Mol Biol* (2012) 933:3–16. doi:10.1007/978-1-62703-068-7_1
262. Fitzpatrick F, Lepault F, Homo-Delarche F, Bach JF, Dardenne M. Influence of castration, alone or combined with thymectomy, on the development of diabetes in the nonobese diabetic mouse. *Endocrinology* (1991) 129(3):1382–90. doi:10.1210/endo-129-3-1382
263. Rosmalen JG, Pigmans MJ, Kersseboom R, Drexhage HA, Leenen PJ, Homo-Delarche F. Sex steroids influence pancreatic islet hypertrophy and subsequent autoimmune infiltration in nonobese diabetic (NOD) and NODscid mice. *Lab Invest* (2001) 81(2):231–9. doi:10.1038/labinvest.3780231
264. Fox HS. Androgen treatment prevents diabetes in nonobese diabetic mice. *J Exp Med* (1992) 175(5):1409–12. doi:10.1084/jem.175.5.1409
265. Toyoda H, Takei S, Formby B. Effect of 5-alpha dihydrotestosterone on T-cell proliferation of the female nonobese diabetic mouse. *Proc Soc Exp Biol Med* (1996) 213(3):287–93. doi:10.3181/00379727-213-44060
266. Bao M, Yang Y, Jun HS, Yoon JW. Molecular mechanisms for gender differences in susceptibility to T cell-mediated autoimmune diabetes in nonobese diabetic mice. *J Immunol* (2002) 168(10):5369–75. doi:10.4049/jimmunol.168.10.5369
267. Markle JG, Frank DN, Mortin-Toth S, Robertson CE, Feazel LM, Rolle-Kampczyk U, et al. Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. *Science* (2013) 339(6123):1084–8. doi:10.1126/science.1233521
268. Gourdy P, Bourgeois EA, Levescot A, Pham L, Riant E, Ahui ML, et al. Estrogen therapy delays autoimmune diabetes and promotes the protective efficiency of natural killer T-cell activation in female nonobese diabetic mice. *Endocrinology* (2016) 157(1):258–67. doi:10.1210/en.2015-1313
269. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* (2004) 27(5):1047–53. doi:10.2337/diacare.27.10.2569-a
270. Geisler JG, Zawalich W, Zawalich K, Lakey JR, Stukenbrok H, Milici AJ, et al. Estrogen can prevent or reverse obesity and diabetes in mice expressing human islet amyloid polypeptide. *Diabetes* (2002) 51(7):2158–69. doi:10.2337/diabetes.51.7.2158
271. Le May C, Chu K, Hu M, Ortega CS, Simpson ER, Korach KS, et al. Estrogens protect pancreatic beta-cells from apoptosis and prevent insulin-deficient diabetes mellitus in mice. *Proc Natl Acad Sci U S A* (2006) 103(24):9232–7. doi:10.1073/pnas.0602956103
272. Contreras JL, Smyth CA, Bilbao G, Young CJ, Thompson JA, Eckhoff DE. 17beta-Estradiol protects isolated human pancreatic islets against proinflammatory cytokine-induced cell death: molecular mechanisms and islet functionality. *Transplantation* (2002) 74(9):1252–9. doi:10.1097/00007890-200211150-00010
273. Mauvais-Jarvis F, Clegg DJ, Hevener AL. The role of estrogens in control of energy balance and glucose homeostasis. *Endocr Rev* (2013) 34(3):309–38. doi:10.1210/er.2012-1055
274. Shen M, Kumar SP, Shi H. Estradiol regulates insulin signaling and inflammation in adipose tissue. *Horm Mol Biol Clin Investig* (2014) 17(2):99–107. doi:10.1515/hmbci-2014-0007
275. Rubinow KB, Chao JH, Hagman D, Kratz M, Van Yserloo B, Gaikwad NW, et al. Circulating sex steroids coregulate adipose tissue immune cell populations in healthy men. *Am J Physiol Endocrinol Metab* (2017) 313(5):E528–39. doi:10.1152/ajpendo.00075.2017
276. Butte NF. Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus. *Am J Clin Nutr* (2000) 71(5 Suppl):1256S–61S. doi:10.1093/ajcn/71.5.1256s
277. Sugaya A, Sugiyama T, Yanase S, Shen XX, Minoura H, Toyoda N. Expression of glucose transporter 4 mRNA in adipose tissue and skeletal muscle of ovariectomized rats treated with sex steroid hormones. *Life Sci* (2000) 66(7):641–8. doi:10.1016/S0024-3205(99)00636-0
278. Straub SG, Sharp GW, Meglasson MD, De Souza CJ. Progesterone inhibits insulin secretion by a membrane delimited, non-genomic action. *Biosci Rep* (2001) 21(5):653–66. doi:10.1023/A:1014773010350
279. Nunes VA, Portioli-Sanches EP, Rosim MP, Araujo MS, Praxedes-Garcia P, Valle MM, et al. Progesterone induces apoptosis of insulin-secreting cells: insights into the molecular mechanism. *J Endocrinol* (2014) 221(2):273–84. doi:10.1530/JOE-13-0202
280. Yu IC, Lin HY, Sparks JD, Yeh S, Chang C. Androgen receptor roles in insulin resistance and obesity in males: the linkage of androgen-deprivation therapy to metabolic syndrome. *Diabetes* (2014) 63(10):3180–8. doi:10.2337/db13-1505
281. Navarro G, Allard C, Xu W, Mauvais-Jarvis F. The role of androgens in metabolism, obesity, and diabetes in males and females. *Obesity (Silver Spring)* (2015) 23(4):713–9. doi:10.1002/oby.21033
282. Dubois V, Laurent MR, Jardi F, Antonio L, Lemaire K, Goyvaerts L, et al. Androgen deficiency exacerbates high-fat diet-induced metabolic alterations in male mice. *Endocrinology* (2016) 157(2):648–65. doi:10.1210/en.2015-1713
283. Xu W, Niu T, Xu B, Navarro G, Schipma MJ, Mauvais-Jarvis F. Androgen receptor-deficient islet β-cells exhibit alteration in genetic markers of insulin secretion and inflammation. A transcriptome analysis in the male mouse. *J Diabetes Complications* (2017) 31(5):787–95. doi:10.1016/j.jdiacomp.2017.03.002
284. Morimoto S, Mendoza-Rodríguez CA, Hiriart M, Larrieta ME, Vital P, Cerbón MA. Protective effect of testosterone on early apoptotic damage induced by streptozotocin in rat pancreas. *J Endocrinol* (2005) 187(2):217–24. doi:10.1677/joe.1.06357
285. Palomar-Morales M, Morimoto S, Mendoza-Rodríguez CA, Cerbón MA. The protective effect of testosterone on streptozotocin-induced apoptosis in beta cells is sex specific. *Pancreas* (2010) 39(2):193–200. doi:10.1097/MPA.0b013e3181c156d9
286. Navarro G, Xu W, Jacobson DA, Wicksteed B, Allard C, Zhang G, et al. Extranuclear actions of the androgen receptor enhance glucose-stimulated insulin secretion in the male. *Cell Metab* (2016) 23(5):837–51. doi:10.1016/j.cmet.2016.03.015
287. Braun M, Ramracheya R, Bengtsson M, Clark A, Walker JN, Johnson PR, et al. Gamma-aminobutyric acid (GABA) is an autocrine excitatory transmitter in human pancreatic beta-cells. *Diabetes* (2010) 59(7):1694–701. doi:10.2337/db09-0797

288. Soltani N, Qiu H, Aleksic M, Glinka Y, Zhao F, Liu R, et al. GABA exerts protective and regenerative effects on islet beta cells and reverses diabetes. *Proc Natl Acad Sci U S A* (2011) 108(28):11692–7. doi:10.1073/pnas.1102715108
289. Tian J, Dang H, Chen Z, Guan A, Jin Y, Atkinson MA, et al. Gamma-aminobutyric acid regulates both the survival and replication of human β-cells. *Diabetes* (2013) 62(11):3760–5. doi:10.2337/db13-0931
290. Beales PE, Hawa M, Williams AJ, Albertini MC, Giorgini A, Pozzilli P. Baclofen, a gamma-aminobutyric acid-b receptor agonist, delays diabetes onset in the non-obese diabetic mouse. *Acta Diabetol* (1995) 32(1):53–6. doi:10.1007/BF00581047
291. He S, Zhang Y, Wang D, Tao K, Zhang S, Wei L, et al. Rapamycin/GABA combination treatment ameliorates diabetes in NOD mice. *Mol Immunol* (2016) 73:130–7. doi:10.1016/j.molimm.2016.01.008
292. Tian J, Lu Y, Zhang H, Chau CH, Dang HN, Kaufman DL. Gamma-aminobutyric acid inhibits T cell autoimmunity and the development of inflammatory responses in a mouse type 1 diabetes model. *J Immunol* (2004) 173(8):5298–304. doi:10.4049/jimmunol.173.8.5298
293. Alam S, Laughton DL, Walding A, Wolstenholme AJ. Human peripheral blood mononuclear cells express GABAA receptor subunits. *Mol Immunol* (2006) 43:1432–42. doi:10.1016/j.molimm.2005.07.025
294. Tian J, Dang HN, Yong J, Chui WS, Dizon MP, Yaw CK, et al. Oral treatment with gamma-aminobutyric acid improves glucose tolerance and insulin sensitivity by inhibiting inflammation in high fat diet-fed mice. *PLoS One* (2011) 6(9):e25338. doi:10.1371/journal.pone.0025338
295. Cavagnini F, Pinto M, Dubini A, Invitti C, Cappelletti G, Polli EE. Effects of gamma aminobutyric acid (GABA) and muscimol on endocrine pancreatic function in man. *Metabolism* (1982) 31(1):73–7. doi:10.1016/0026-0495(82)90029-4
296. Passariello N, Giugliano D, Torella R, Sgambato S, Coppola L, Frascolla N. A possible role of gamma-aminobutyric acid in the control of the endocrine pancreas. *J Clin Endocrinol Metab* (1982) 54(6):1145–9. doi:10.1210/jcem-54-6-1145
297. Yoon JW, Yoon CS, Lim HW, Huang QQ, Kang Y, Pyun KH, et al. Control of autoimmune diabetes in NOD mice by GAD expression or suppression in beta cells. *Science* (1999) 284(5417):1183–7. doi:10.1126/science.284.5417.1183
298. Ryden AK, Wesley JD, Coppieters KT, Von Herrath MG. Non-antigenic and antigenic interventions in type 1 diabetes. *Hum Vaccin Immunother* (2014) 10(4):838–46. doi:10.4161/hv.26890
299. O'Mahony L, Akdis M, Akdis CA. Regulation of the immune response and inflammation by histamine and histamine receptors. *J Allergy Clin Immunol* (2011) 128(6):1153–62. doi:10.1016/j.jaci.2011.06.051
300. Jutel M, Watanabe T, Klunker S, Akdis M, Thomet OA, Malolepszy J, et al. Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. *Nature* (2001) 413(6854):420–5. doi:10.1038/35096564
301. Frewin DB, Cleland LG, Jonsson JR, Robertson PW. Histamine levels in human synovial fluid. *J Rheumatol* (1986) 13(1):13–4.
302. Gill DS, Barradas MA, Fonseca VA, Dandona P. Plasma histamine concentrations are elevated in patients with diabetes mellitus and peripheral vascular disease. *Metabolism* (1989) 38(3):243–7. doi:10.1016/0026-0495(89)90082-6
303. Tuomisto L, Kilpeläinen H, Riekkinen P. Histamine and histamine-N-methyltransferase in the CSF of patients with multiple sclerosis. *Agents Actions* (1983) 13(2–3):255–7. doi:10.1007/BF01967346
304. Alkan M, Machavoine F, Rignault R, Dam J, Dy M, Thieblemont N. Histidine decarboxylase deficiency prevents autoimmune diabetes in NOD mice. *J Diabetes Res* (2015) 2015:965056. doi:10.1155/2015/965056
305. Yoshimoto R, Miyamoto Y, Shimamura K, Ishihara A, Takahashi K, Kotani H, et al. Therapeutic potential of histamine H3 receptor agonist for the treatment of obesity and diabetes mellitus. *Proc Natl Acad Sci U S A* (2006) 103(37):13866–71. doi:10.1073/pnas.0506104103
306. Takahashi K, Suwa H, Ishikawa T, Kotani H. Targeted disruption of H3 receptors results in changes in brain histamine tone leading to an obese phenotype. *J Clin Invest* (2002) 110(12):1791–9. doi:10.1172/JCI15784
307. Masaki T, Yoshimatsu H, Chiba S, Watanabe T, Sakata T. Targeted disruption of histamine H1-receptor attenuates regulatory effects of leptin on feeding, adiposity, and UCP family in mice. *Diabetes* (2001) 50(2):385–91. doi:10.2337/diabetes.50.2.385
308. Fülöp AK, Földes A, Buzás E, Hegyi K, Miklós IH, Romics L, et al. Hyperleptinemia, visceral adiposity, and decreased glucose tolerance in mice with a targeted disruption of the histidine decarboxylase gene. *Endocrinology* (2003) 144(10):4306–14. doi:10.1210/en.2003-0222
309. Masaki T, Chiba S, Yasuda T, Noguchi H, Kakuma T, Watanabe T, et al. Involvement of hypothalamic histamine H1 receptor in the regulation of feeding rhythm and obesity. *Diabetes* (2004) 53(9):2250–60. doi:10.2337/diabetes.53.9.2250
310. Anvari E, Fred RG, Welsh N. The H1-receptor antagonist cetirizine protects partially against cytokine- and hydrogen peroxide-induced β-TC6 cell death in vitro. *Pancreas* (2014) 43(4):624–9. doi:10.1097/MPA.0000000000000076
311. Anvari E, Wang X, Sandler S, Welsh N. The H1-receptor antagonist cetirizine ameliorates high-fat diet-induced glucose intolerance in male C57BL/6 mice, but not diabetes outcome in female non-obese diabetic (NOD) mice. *Ups J Med Sci* (2015) 120(1):40–6. doi:10.3109/03009734.2014.967422

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 01 May 2018
doi: 10.3389/fendo.2018.00214

Tuberculosis, the Disrupted Immune-Endocrine Response and the Potential Thymic Repercussion As a Contributing Factor to Disease Physiopathology

*Luciano D'Attilio, Natalia Santucci, Bettina Bongiovanni, María L. Bay and Oscar Bottasso**

Instituto de Inmunología Clínica y Experimental de Rosario, UNR-CONICET, Rosario, Argentina

OPEN ACCESS

Edited by:
Daniella Areas Mendes-Da-Cruz, Fundação Oswaldo Cruz (Fiocruz), Brazil

Reviewed by:
Aldo Tagliabue, Istituto di Ricerca Genetica e Biomedica (CNR), Italy
Bruno R-s, Unidad de Investigación Biomédica de Zacatecas (IMSS), Mexico

***Correspondence:**
Oscar Bottasso
bottasso@idicer-conicet.gob.ar

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: *23 November 2017*
Accepted: *16 April 2018*
Published: *01 May 2018*

Citation:
D'Attilio L, Santucci N, Bongiovanni B, Bay ML and Bottasso O (2018) Tuberculosis, the Disrupted Immune-Endocrine Response and the Potential Thymic Repercussion As a Contributing Factor to Disease Physiopathology. Front. Endocrinol. 9:214. doi: 10.3389/fendo.2018.00214

Upon the pathogen encounter, the host seeks to ensure an adequate inflammatory reaction to combat infection but at the same time tries to prevent collateral damage, through several regulatory mechanisms, like an endocrine response involving the production of adrenal steroid hormones. Our studies show that active tuberculosis (TB) patients present an immune-endocrine imbalance characterized by an impaired cellular immunity together with increased plasma levels of cortisol, pro-inflammatory cytokines, and decreased amounts of dehydroepiandrosterone. Studies in patients undergoing specific treatment revealed that cortisol levels remained increased even after several months of initiating therapy. In addition to the well-known metabolic and immunological effects, glucocorticoids are involved in thymic cortical depletion with immature thymocytes being quite sensitive to such an effect. The thymus is a central lymphoid organ supporting thymocyte T-cell development, i.e., lineage commitment, selection events and thymic emigration. While thymic TB is an infrequent manifestation of the disease, several pieces of experimental and clinical evidence point out that the thymus can be infected by mycobacteria. Beyond this, the thymic microenvironment during TB may be also altered because of the immune-hormonal alterations. The thymus may be then an additional target of organ involvement further contributing to a deficient control of infection and disease immunopathology.

Keywords: tuberculosis, immune-endocrine communication, inflammation, thymic involution, pathophysiology, hormones

TUBERCULOSIS (TB) AND ITS MAIN PATHOPHYSIOLOGICAL FEATURES

Mycobacterium tuberculosis (*M. tuberculosis*), the etiologic agent of TB, is responsible for more deaths worldwide than any single pathogen with an estimated 10.4 million patients and 1.3 million deaths, annually in 2016 (1). Most cases of primary TB infection are clinically and radiologically unapparent. These individuals remain persistently infected by *M. tuberculosis* constituting non-contagious carriers of the bacillus but setting the stage for subsequent reappearance.

About 5% of patients pass from latency to post-primary disease within 2 years of primary infection and another 5% do so in later lives. While most cases of post-primary TB in immunocompetent adults arise from reactivation from latent infection, molecular studies showed that exogenous reinfection accounts for a significant percentage of cases in some areas of the world. Adult post-primary TB typically affects the best aerated lung regions, preferably the upper lobes (2, 3). The histopathological hallmark is a granuloma composed of epithelioid cells with variable numbers of Langhans' giant cells surrounded by lymphocytes and a central zone of caseation necrosis and variable degree of fibrosis (3–6). The structure is surrounded by a fibrous capsule which constitutes a contention barrier. A spectrum of lesions may be seen from a hard granuloma without necrosis and rare organisms to the one with multibacillary necrotic lesions in the central zone, even within the same patient (7, 8).

Human infection with *M. tuberculosis* can result in a varied degree of organic compromise, ranging from an asymptomatic process to frank lung pathology with cavity formation and high bacillary load. Such clinical spectrum relies on a complex series of interactions between *M. tuberculosis* and the host immune response (4). The defensive reactions mainly involve the microbicidal effect of activated macrophages and the capacity of cytotoxic lymphocytes to destroy infected macrophages. Upon phagocytosis macrophages can produce or receive the influence of different cytokines rendering them more effective in suppressing bacillary replication and possibly destruction of the *mycobacterium*, i.e., IFN-γ (4, 9). This cytokine is secreted primarily by T lymphocytes, particularly the so-called Th1 cells which are involved in the protective immunity toward the mycobacteria (2), although in some circumstances Th1 immunity can also result in unbalanced pulmonary inflammation (9). Possibly, a better correlate of protection deals with the profile of cytokine production, since patients with TB disease showed elevated frequencies of *M. tuberculosis*-specific CD4 T cells expressing only TNF-α or TNF-α^+IFN-γ^+CD4$^+$ T cells, whereas cases with latent TB infection showed greater frequencies of polyfunctional TNFα^+IFN-γ^+IL-2$^+$ *M. tuberculosis* specific CD4$^+$ T cells (10–12).

In our laboratory, we have shown that patients with mild forms of TB have a suitable Th1 response pattern and that it is gradually reduced as the disease progresses (13, 14).

The other mechanism involved in protection comprises the elimination of infected macrophages by cytotoxic lymphocytes through the classical events of granules containing perforin and granzymes or the induction of apoptosis through the Fas-FasL interaction. Following the formation of apoptotic bodies, they are ingested by phagocytes *via* the efferocytosis. The efferosome surrounds the newly incorporated apoptotic cell followed by successive events of fusion with lysosomes, delivery of hydrolytic enzymes to this efferosome in maturation and gradual increase of its acidification to finally proceed with the destruction of apoptotic cells (15). Nevertheless, an increased apoptosis may sometimes spread the infection to neighboring macrophages considering the extensive apoptosis seen within caseating granulomas of patients with lung TB (16).

THE ALTERED IMMUNE-ENDOCRINE COMMUNICATION IN TB

Tuberculosis constitutes a natural model wherein the essential processes required for mounting successful defensive strategies and homeostasis maintenance may result detrimental when the infection becomes chronic, as the accompanying inflammation. Our studies point out that such disorder not only affects the containment mechanisms but also the immune-endocrine communication, favoring a more morbid disease course (17).

The bidirectional communication between the neuroendocrine and immune systems is well-known. While products from the immune response can modify the functioning of the endocrine system, hormones like adrenal steroids directly affect the activity of immune cells and hence the course of disease-states with an inflammatory, autoimmune, or infectious background. This interconnection between the immune and the neuroendocrine systems is partly due to the stimulatory activity of inflammatory cytokines on the hypothalamus pituitary adrenal (HPA) axis. Briefly, cytokines such as IL-6, IL-1β, and TNF-α stimulate the production of corticotropin-releasing hormone (CRH) in the hypothalamus with subsequent release of adrenocorticotrophin into the pituitary gland, which in turn promotes the secretion of steroid hormones at the level of the adrenal cortex: cortisol and dehydroepiandrosterone (DHEA) (18, 19). Both hormones are known to exert relevant immunomodulatory effects. For instance, glucocorticoids (GCs) can inhibit Th1 responses, whereas their natural antagonist DHEA is able to favor them (18, 19). As part of integrated physiological circuits, these endocrine reactions, particularly the HPA axis, represent a well-conserved mechanism to control/support an intense immune-inflammatory reaction as well as for the early mobilization of immune cells and their redistribution to mount an adequate defensive response. Nevertheless, when the inflammatory condition becomes persistent such prolonged immuno-inflammatory aggression leads to a misuse of these evolutionarily conserved control mechanisms contributing to exacerbate host damage (20, 21).

Regrettably, the implication of these reciprocities in the field of pathogenesis, prognosis and treatment of chronic infectious diseases remains underestimated.

Beyond inhibiting the development Th1 cells in favor of Th2 responses (22, 23), GCs also interfere with gene expression for pro-inflammatory cytokines, by hindering nuclear factor kappa B (NF-κB) signaling (24, 25). More recent studies reveal that during the immune response GCs exert differential effects on effector and regulatory T cells with an intense inhibition in the proliferation of the former and a differential apoptosis of the latter (26). Under certain conditions, GCs may also have pro-inflammatory effects by some not well characterized mechanism. These apparently opposing actions would work together to prepare the immune system to respond to the stressful stimulus (pro-inflammatory effect) and subsequently to restore homeostasis—an anti-inflammatory effect—which is obviously the most prominent role of GCs (27). On its own, DHEA is also able to inhibit the secretion of pro-inflammatory cytokines such as IL-6 and TNF-α (28, 29).

To ascertain the immunoendocrine alterations during TB, we initially studied the circulating levels of cytokines and hormones such as IFN-γ, IL-10, IL-6, cortisol, DHEA, GH in male TB patients with different degrees of lung involvement and free from endocrine disorders, or treatment with corticosteroids or immunomodulatory drugs. Patients presented increased levels of IL-6, IFN-γ, and cortisol, whereas DHEA levels were well below the control values, the lowest levels corresponding to those with advanced disease (30). In line with this, other studies in active TB patients from Turkey and South Africa also revealed decreased DHEA levels (31–33), whereas cortisol concentrations appeared unchanged (31, 32) or slightly increased (33).

At the *in vitro* level, treatment of peripheral blood mononuclear cells (PBMCs) with cortisol, at slightly supraphysiological levels, resulted in a decreased proliferation and production of IFN-γ to mycobacterial antigen stimulation, with no changes in IL-10 production (34). DHEA, on its own, caused a significant decrease in the production of TGF-β by PBMCs of patients with advanced TB (34), a cytokine which is well known for its suppressive and harmful effects on TB (17). When studying the functional capacity of dendritic cells exposed to *M. tuberculosis* antigens, cortisol significantly inhibited the secretion of IL-12, IFN-γ, and IL-10 by these cells, whereas DHEA increased the expression of MHC-I, MHC-II, and CD86, in addition to improving IL-12 production and decreasing IL-10 secretion (35). DHEA also inhibited the intra-macrophage bacillary growth, which was related to a higher level of autophagy (36). Collectively, our studies are consistent with the view of a respective detrimental or favorable influence of cortisol and DHEA on the anti-TB immune response.

As part of this interrelation between the endocrine system and the immune system, culture supernatants of PBMCs from TB patients stimulated with mycobacterial antigens inhibited the secretion of DHEA by the human adrenal cell line NCI-H295-R (30) whereas treatment with anti-TGF-β neutralizing antibodies reversed this inhibitory effect (37). This observation reinforces the close network of influences underlying immunoendocrine regulation, particularly the production level of adrenal steroids and immune mediators.

Changes in the immune-endocrine communication may be also implicated in situations further contributing to disease morbidity. In fact, we have demonstrated that the defective *in vitro* immune responses of TB patients to mycobacterial antigens was related to their reduced body mass index (BMI), which was negatively correlated with IL-6 circulating levels (38). This cytokine is known to play a role in the regulation of lipid metabolism and studies in TB patients indicate that increased IL-6 concentrations were associated with loss of appetite (39). Regarding hormones, GCs may favor a loss of body mass since they mobilize lipid stores by inducing lipolysis in fat cells *via* stimulation of a hormone-sensitive lipoprotein lipase. Also, GCs inhibit protein synthesis and stimulate proteolysis in muscle cells (40), in addition to reducing food intake and inducing body weight loss, probably *via* increased hypothalamic CRH levels, which seems to be catabolic (41). In essence, the immune-endocrine profile is adverse for the patient being involved in the reduction of body weight or consumption state during infections. This situation, defined as cachexia is a multifaceted metabolic disturbance present in several chronic inflammatory diseases or end stage neoplasms comprising weight loss, adipose tissue and skeletal muscle depletion, along with reduced appetite. The mechanisms underlying cachexia development are complex, encompassing the participation of neurologic, metabolic, immunologic, and endocrinological factors (42–44). In this context, we have recently found that the lower BMI of patients coexists with reduced levels of leptin, whereas concentrations of IL-6, cortisol, IL-1β, and adiponectin were increased (45).

The basis for the above described alterations has to do with the acute phase response (46), an adaptive reaction trying to be beneficial for the host at least during the early infection (46). This leads to a new metabolic set point attempting to attain an optimal functioning of the immunological needs, without affecting requirements of some often-competing physiological functions (47, 48). Since energy is not a limitless resource, when the infection becomes chronic metabolic deficit establishes further affecting the defensive reaction and disease outcome.

The link between energy supply and the immune response is supported from a study carried out in Africa in which the metabolic needs to cope with measles further impaired body weight in undernourished children (49). In turn, malnutrition may also affect the immune response through hormonal influences, given the respective reduced and increased leptin and GC levels in undernourished persons (50, 51). In addition to the inhibitory effects of GCs on cell-mediated immune responses (52, 53), leptin also displays immunostimulating effects (54, 55). Leptin deficient animals show atrophy of lymphoid organs, mainly the thymus, which can be reversed upon the leptin administration (56). Accordingly, it may be assumed that the consumption state of TB patients along with the decreased or increased leptin and GCs levels may impact negatively on thymus function.

THYMUS INVOLVEMENT IN TB, FACTS, AND HYPOTHESIS

Because of the continuous need to replenish mature peripheral T cells that undergo normal turnover throughout life (57), preserved thymus during *M. tuberculosis* infection in the mammalian host may be essential for the development of an effective immune response against mycobacteria.

Animal studies showed that following erogenic infection, the thymus is as likely to be infected with *M. tuberculosis* as the lung tissue (58). Thymic compromise may be observed in bacterial infections, including those caused by mycobacteria, i.e., *M. tuberculosis* and *M. avium* (59–62). Despite some immunological compromise, thymus infection also displays compensatory strategies aimed at improving thymic function; that is the identification of *Mycobacterium*-reactive T cells within the thymus that migrated from the peripheral compartment (63, 64).

As regards to the clinical field, while historical histopathological preparations from old patients identified the occurrence of thymic TB (65) thymic TB is an infrequent presentation of the disease, with a bit more of a dozen cases being reported in the literature (66, 67).

Without being mutually exclusive, it can be assumed that the endocrine abnormalities present in TB may also affect the thymus by mechanisms that go beyond the infection *per se*, resulting equally detrimental, i.e., a deficient immune competence or thymic selection. In normal conditions, bone marrow T-cell progenitors migrate to the thymus to undergo a broad process of differentiation and selection. Thymocyte positive selection is mediated by thymic epithelial cells (TECs), which not only display antigen-presenting activity, but also secrete compounds or express cell surface molecules essential for thymocyte development. In the medulla, medullary TECs allow the T-cell recognition of self-antigens by facilitating the expression of tissue-related antigens and presenting them to developing thymocytes. Central T-cell tolerance also takes place in the thymic medulla, for which the removal of harmful and autoreactive T-cell clones is achieved (68–70). After entering the thymus, thymocytes representing different stages of development occupy distinct regions of the thymus. Thymocyte progenitors referred to as double negative cells ($CD3^-CD4^-CD8^-$) locate at the cortico-medullary junction, where undergo rapid proliferation, mostly driven by IL-7, and further migrate through the cortex toward the medulla. Cells unable to rearrange their antigen receptor genes will endure apoptosis, whereas those experiencing gene rearrangements of the T-cell receptor genes and acquisition of both CD4 and CD8 coreceptors ($CD4^+CD8^+$ double positive—DP cells) undergo positive (functional TCR) and negative (non self-reactive TCR) selection in the cortex and medulla. Most DP cells have nonfunctional antigen receptors rendering them unable to receive surviving signals for which they undergo apoptosis (death by neglect). The surviving cells, which loss either CD4 or CD8 molecules and become single positive (SP) cells, undergo negative selection; that is an activation-induced cell death of cells with high affinity antigen receptor for self-antigens. Finally, cells leave the thymus as $CD4^-CD8^+$ (cytotoxic) or $CD4^+CD8^-$ (helper), SP mature, naïve T cells (68–70).

Turning to the disturbed immune-endocrine responses seen in TB patients there is reason to believe that such changes, particularly the ones dealing with adrenal steroids and leptin may indirectly compromise thymus function, favoring gland involution. Thymic involution is the progressive loss of the thymus to sustain lymphopoiesis and the ensuing impairment for *de novo* T-cell production. Thymic senescence starts well advanced puberty and by 50 years of age 80% of the thymic stroma is replaced by adipose tissue. The maximum decline in the thymic weight occurs between 30 and 40 years of age (71, 72), which might account for some evidence of a lower thymic activity seen in individuals older than 40–50 years (73, 74).

Besides aging, thymic involution can be provoked by several conditions and factors: among them pregnancy, severe infections, cancer, irradiation and hormones, like GCs (70). In mouse models, high doses of GCs cause thymocyte depletion, involving especially DP thymocytes and TECs (70, 75). Some experimental studies also suggest that GC production at the thymic level may influence thymocyte differentiation and thymic homeostasis (76–78).

According to the neuroendocrine influence on thymic function, infectious diseases and the malnutrition state that may accompany in some cases, i.e., TB, are quite likely to affect thymic activity (79, 80).

Although at the experimental level low GCs concentrations may rescue thymocytes from the TCR-mediated apoptosis (81, 82), the scenario in TB patients is characterized by a chronic elevation of cortisol that while being of moderate intensity remained so even after several months of treatment initiation (83). Furthermore, TB patients also present quite reduced amounts of circulating leptin levels (45). This hormone prevents starvation-induced thymic atrophy (84) along with a protective effect on the loss of lymphoid and TEC populations occurring in the stress-induced acute atrophy of the thymus (85). It follows that increased cortisol and reduced leptin levels promote an unsuitable scenario for a proper thymus function.

Our study in TB patients showed decreased levels of testosterone and DHEA, in presence of augmented amounts of GH, not accompanied by increased IGF-1 levels, in parallel to modest increases estradiol, prolactin (PRL), and thyroid hormones (30) (a summary of immune-endocrine alterations is provided in **Figure 1**).

Pretreatment of mice with DHEA was found to result in a partial protection from the GC-induced decrease in thymus weight and thymocyte death (86, 87). Similarly, administration of DHEA to male mice partially or completely reversed the dexamethasone-inhibited blastogenic response to mitogen stimulation (88). Depending on the experimental conditions, *in vitro* treatment with DHEA may promote thymocyte apoptosis (89) or even exert an anti-apoptotic effect on these cells (90). Studies in rats undergoing a repeated immobilization stress showed that DHEA behaved as an anti-stress hormone (91), whereas DHEA supplementation in rats undergoing an experimental *Trypanosoma cruzi* infection led to an improved thymocyte proliferation and reduced TNF-α production (92). Collectively, these findings tip the balance toward a favorable role of DHEA on thymus function, for which reduced levels of DHEA in TB patients may be also disadvantageous. Hormones other than the HPA axis are also likely to influence the thymus gland [reviewed in Ref. (71)]. GH is known to increase the release of cytokines, chemokines and thymulin (93), and to augment the deposition of proteins implicated in cell migration (94, 95); whereas PRL facilitates the survival and proliferation of early T–cell progenitors (96). Aged rat recipients of cells from a pituitary adenoma secreting GH and PRL appeared recovered from the thymic involution (97), as well. The extent to which GH may be operative in our patient series is uncertain since increased GH levels were not accompanied by an increase in IGF-1 implying a state of resistance to GH (30). About PRL, the increase seen in TB patients was quite low as did thyroid hormones (30). In situations of greater exposure thyroid hormones may be beneficial as seen in T3-treated mice (98) or the relation between hyperthyroidism with thymic hyperplasia because of the increased numbers of thymocytes (99). Since the increase in thyroid hormones of TB patients did not fit with a clear hyperthyroidism, we remain unsure on the role of these hormones on the thymic gland.

Some pieces of evidence point out that sex steroid have deleterious effects at the thymic level since thymus atrophy

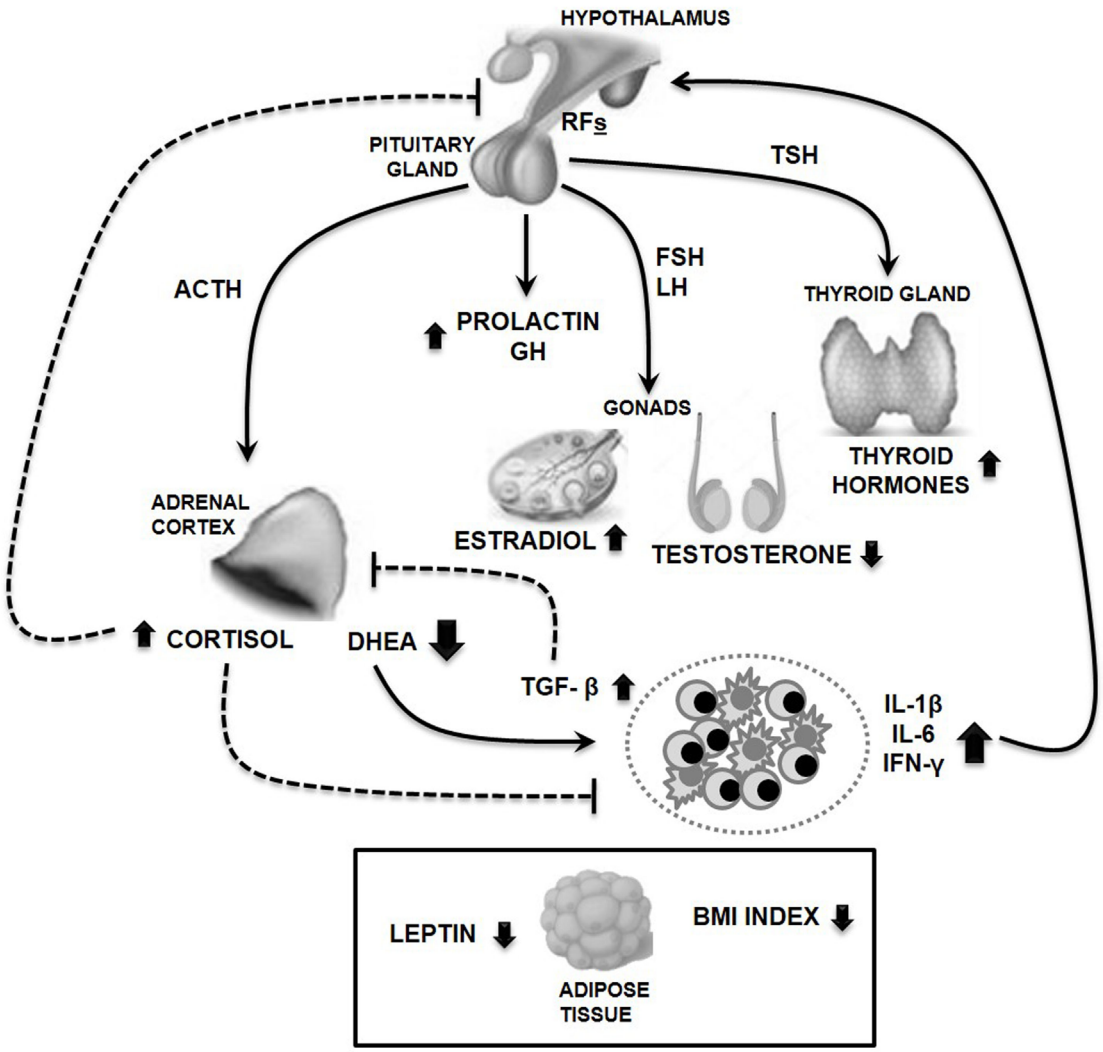

FIGURE 1 | Main features of circulating immune-endocrine alterations in male tuberculosis (TB) patients. Cytokine release by immunocompetent cells stimulates the production of releasing factors (RFs) at the hypothalamic levels, like the corticotropin-releasing hormone leading to the pituitary synthesis of adrenocorticotropin hormone (ACTH). This is followed by the production of adrenal steroids, cortisol, and dehydroepiandrosterone (DHEA), which are, respectively, increased or decreased during TB. Such unbalanced cortisol/DHEA relation along with the altered production of gonadal steroids are much likely to favor a Th1→Th2 immune shift, further accompanied by reduced amounts of leptin, an immunostimulating compound. Presence of transforming growth factor beta (TGF-β) which is increased in TB, in turn, inhibits DHEA production by adrenal cells. TB patients also displayed increased amounts of growth hormone (GH) and prolactin probably related to the protracted inflammation, in addition to augmented levels of thyroid hormones. This pattern of hormonal alterations would favor a deficient infection control together with a catabolic status, as exemplified by the reduced body mass index (BMI) and leptin plasma levels seen in patients (represented in a separate box dealing with a metabolic component). Solid and dashed lines represent stimulating and inhibiting effects, respectively. Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone; TSH, thyroid-stimulating hormone; IL-6, interleukin 6; IL-1β, interleukin 1 beta; IFN-γ, interferon gamma.

accelerates at puberty (100), whereas administration of androgens or estrogens in adult mice results in a remarkably decreased thymopoiesis linked to an increased apoptosis of cortical thymocytes (101). In our study, testosterone and estradiol were comparatively decreased or increased, respectively (30), for which the thymic role of both steroids in the TB scenario remains uncertain.

Collectively, the evidence discussed indicates a harmful influence of immune-endocrine alterations at the level of the thymus; however, these changes may be reversible and associated with the clinical improvement of patients, leading to an eventual normalization of the thymic function.

The scenario present in TB patients can be conciliated with the view wherein neuroendocrine hormones released in response

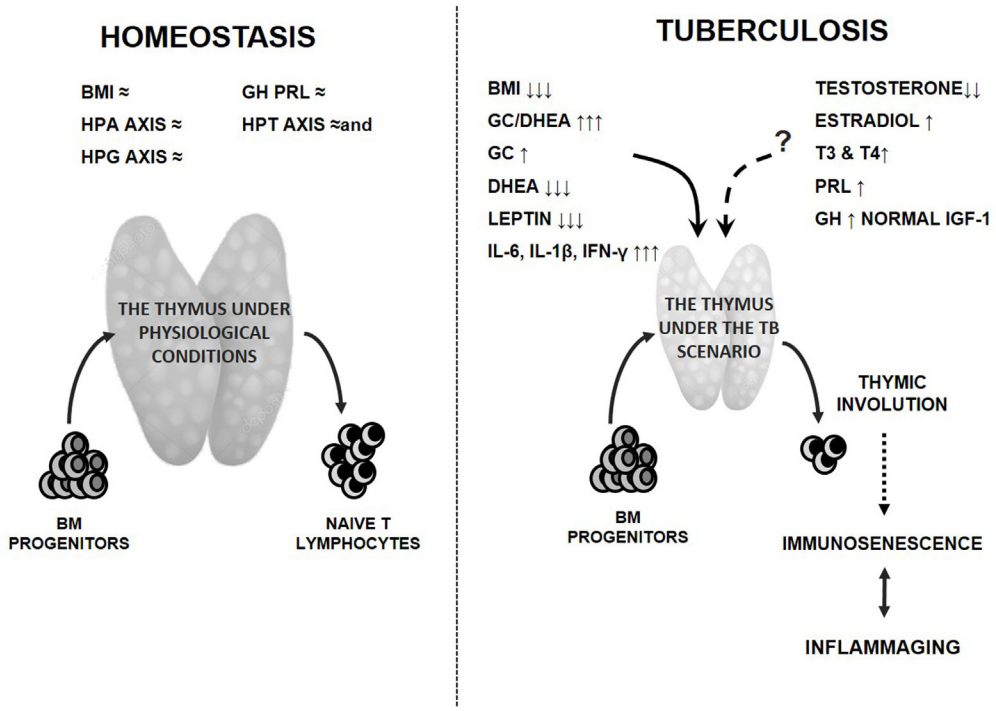

FIGURE 2 | Endocrine alterations in tuberculosis (TB) patients and the potential thymic repercussion. Detrimental effects of clinical and endocrine disturbances on the thymus gland and function during TB are presented by solid lines, which is the consumption state along with the increased amounts of cortisol and pro-inflammatory cytokines in presence of reduced levels of leptin and DHEA. While administration of androgens or estrogens in adult mice leads to a decreased thymopoiesis, the thymic influence of gonadal steroids in TB is uncertain, since patients displayed decreased or increased levels of testosterone and estradiol, respectively (dashed line). Levels of prolactin and thyroid hormones appeared augmented, but their increases did not reach the values able to mediate a clear beneficial effect on the thymus gland (dashed line). The extent to which GH may be favorable at the thymic level remains also unclear since its increased amounts were not accompanied by higher IGF-1 values compatible with state of GH resistance (dashed line). The resulting thymic involution mostly because of leptin and adrenal steroid changes together with a chronic inflammatory state are likely to lead to premature immunosenescence (dotted line) and the coexisting inflammaging. Most of these changes would contribute to worsen the disease course. The left panel represents the preserved (≈) homeostatic situation. Abbreviations: BM, bone marrow; BMI, body mass index; HPA, hypothalamic pituitary adrenal; HPG, hypothalamic pituitary gonadal; HPT, hypothalamic pituitary thyroid axes; GH, growth hormone; PRL, prolactin; GC, glucocorticoids; DHEA, dehydroepiandrosterone; IGF-1, insulin growth factor like 1; T3, triiodothyronine; T4, thyroxine; IL-6, interleukin 6; IL-1β, interleukin 1 beta; IFN-γ, interferon gamma.

to psychosocial stress, chronic inflammation or persistent infections are likely to result in premature immunosenescence (102), particularly when considering the resemblance of immune changes seen during aging or chronic GC exposure. In fact, the immunosenescence pattern seen in healthy aging is comparable to the one observed in subjects under chronic stress or chronically exposed to GCs, i.e., thymic involution, declined thymic exportation of naive T cells, a Th1→Th2 cytokine shift, increased circulating levels of pro-inflammatory markers and shorter telomere lengths, compatible with an accelerated aging [reviewed in Ref. (103)].

Notably, senescent cells remain metabolically active for which they may influence other cells through a process termed senescence-associated secretory phenotype (104, 105). That is, the secretions of several inflammatory mediators that exacerbate senescence in the same cell or propagate to the neighbor ones or even systemically amplifying a phenomenon termed inflammaging. Many tissues and cell types participate in producing pro- and anti-inflammatory stimuli dealing with Inflammaging (106). The basis for the establishment of age-related diseases involves an excessive production of pro-inflammatory mediators coupled to an inefficient anti-inflammatory reaction (107). Immunosenescence on its own affects both innate and adaptive immunity, thus providing a contributory mechanism to account for an increased morbidity (108–110).

A summary of the immune-endocrine alterations encompassing TB and their eventual repercussion on thymic function is provided in **Figure 2**.

CONCLUDING REMARKS

Tuberculosis is a disease wherein the immune response cannot cope with mycobacteria for which the infection becomes chronic as did the accompanying immuno-inflammatory state. Such situation set the basis for the establishment of an altered immune-endocrine response that will not only impact on the clinical and metabolic status of patients but also on innate and adaptive immune responses. The bulk of evidence discussed here also suggests a still not envisaged view in the sense that immune-endocrine abnormalities, particularly the unbalanced

relationship between adrenal steroids along with decreased leptin levels, in a pro-inflammatory milieu, are much likely to impact adversely on thymic function.

Tuberculosis has taught us a great deal in relation to the physiopathogenesis which take place in the context of human infections and chronic inflammation, not least in identifying the complex networks of events underlying the clinical disease manifestation. Despite such successes much remains to be accomplished. Importantly future studies are needed to appraise the extent of thymic affectation during active disease, the eventual repercussion on the immunological status of patients, mainly in the context of progressive disease, multidrug-resistant TB, or HIV coinfection. An elucidation of these novel pathogenic avenues will lead ultimately to the development of better diagnostic or therapeutic tools facilitating a more integral strategy for disease control.

AUTHOR CONTRIBUTIONS

LD, NS, BB, MB, and OB conceived, designed, and performed the studies serving to prepare the review. LD, NS, BB, MB, and OB wrote the paper.

FUNDING

This work was partly supported by grants from FONCYT (PICT 2016-0279).

REFERENCES

1. World Health Organization. *Global Tuberculosis Report 2017*. Geneva, Switzerland: WHO. Available from: http://www.who.int/tb/publications/global_report/en/ (Accessed: March, 2018).
2. Lyon SM, Rossman MD. Pulmonary tuberculosis. *Microbiol Spectr* (2017) 5:TNMI7–0032. doi:10.1128/microbiolspec.TNMI7-0032-2016
3. Ndlovu H, Marakalala MJ. Granulomas and inflammation: host-directed therapies for tuberculosis. *Front Immunol* (2016) 7:434. doi:10.3389/fimmu.2016.00434
4. O'Garra A, Redford PS, Mcnab FW, Bloom CI, Wilkinson RJ, Berry MP. The immune response in tuberculosis. *Annu Rev Immunol* (2013) 31:475–527. doi:10.1146/annurev-immunol-032712-095939
5. Orme IM, Basaraba RJ. The formation of the granuloma in tuberculosis infection. *Semin Immunol* (2014) 26:601–9. doi:10.1016/j.smim.2014.09.009
6. Guirado E, Schlesinger LS. Modeling the *Mycobacterium tuberculosis* granuloma – the critical battlefield in host immunity and disease. *Front Immunol* (2013) 4:98. doi:10.3389/fimmu.2013.00098
7. Hunter RL. Pathology of post primary tuberculosis of the lung: an illustrated critical review. *Tuberculosis (Edinb)* (2011) 91:497–509. doi:10.1016/j.tube.2011.03.007
8. Hunter RL. Tuberculosis as a three-act play: a new paradigm for the pathogenesis of pulmonary tuberculosis. *Tuberculosis (Edinb)* (2016) 97:8–17. doi:10.1016/j.tube.2015.11.010
9. Mourik BC, Lubberts E, de Steenwinkel JEM, Ottenhoff THM, Leenen PJM. Interactions between type 1 interferons and the Th17 response in tuberculosis: lessons learned from autoimmune diseases. *Front Immunol* (2017) 8:294. doi:10.3389/fimmu.2017.00294
10. Day CL, Abrahams DA, Lerumo L, Janse van Rensburg E, Stone L, O'rie T, et al. Functional capacity of *Mycobacterium tuberculosis* specific T cell responses in humans is associated with mycobacterial load. *J Immunol* (2011) 187:2222–32. doi:10.4049/jimmunol.1101122
11. Harari A, Rozot V, Bellutti Enders F, Perreau M, Stalder JM, Nicod LP, et al. Dominant TNF-α+ *Mycobacterium tuberculosis*-specific CD4+ T cell responses discriminate between latent infection and active disease. *Nat Med* (2011) 17:372–6. doi:10.1038/nm.2299
12. Riou C, Gray CM, Lugongolo M, Gwala T, Kiravu A, Deniso P, et al. A subset of circulating blood mycobacteria-specific CD4 T cells can predict the time to *Mycobacterium tuberculosis* sputum culture conversion. *PLoS One* (2014) 9(7):e102178. doi:10.1371/journal.pone.0102178
13. Dlugovitzky D, Bay ML, Rateni L, Urízar L, Rondelli CF, Largacha O, et al. *In vitro* synthesis of interferon-γ, interleukin-4, transforming growth factor-β, and interleukin 1-β by peripheral blood mononuclear cells from tuberculosis patients. Relationship with the severity of pulmonary involvement. *Scand J Immunol* (1999) 49:210–7. doi:10.1046/j.1365-3083.1999.00492.x
14. Dlugovitzky D, Bay ML, Rateni L, Fiorenza G, Vietti L, Farroni MA, et al. Influence of disease severity on nitrite and cytokine production by peripheral blood mononuclear cells from patients with pulmonary tuberculosis. *Clin Exp Immunol* (2000) 122:343–9. doi:10.1046/j.1365-2249.2000.01394.x
15. Martin CJ, Peters KN, Behar SM. Macrophages clean up: efferocytosis and microbial control. *Curr Opin Microbiol* (2014) 17:17–23. doi:10.1016/j.mib.2013.10.007
16. Keane J, Balcewicz-Sablinska MK, Remold HG, Chupp GL, Meek BB, Fenton MJ, et al. Infection by *Mycobacterium tuberculosis* promotes human alveolar macrophage apoptosis. *Infect Immun* (1997) 65:298–304.
17. Bottasso O, Bay ML, Besedovsky H, del Rey A. Adverse neuro-immune-endocrine interactions in patients with activetuberculosis. *Mol Cell Neurosci* (2013) 53:77–85. doi:10.1016/j.mcn.2012.11.002
18. Besedovsky H, del Rey A. Immune-neuro-endocrine interactions: facts and hypothesis. *Endocr Rev* (1996) 17:64–95. doi:10.1210/edrv-17-1-64
19. Turnbull AV, Rivier CL. Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. *Physiol Rev* (1999) 79:1–71. doi:10.1152/physrev.1999.79.1.1
20. Straub RH, Schuld A, Mullington J, Haack M, Schölmerich J, Pollmächer T. The endotoxin-induced increase of cytokines is followed by an increase of cortisol relative to dehydroepiandrosterone (DHEA) in healthy male subjects. *J Endocrinol* (2002) 175:467–74. doi:10.1677/joe.0.1750467
21. Cutolo M, Wilder RL. Different roles for androgens and estrogens in the susceptibility to autoimmune rheumatic diseases. *Rheum Dis Clin North Am* (2000) 26:825–39. doi:10.1016/S0889-857X(05)70171-X
22. Hu X, Li WP, Meng C, Ivashkiv LB. Inhibition of IFN-γ signaling by glucocorticoids. *J Immunol* (2003) 170:4833–9. doi:10.4049/jimmunol.170.9.4833
23. Wiegers GJ, Reul JM. Induction of cytokine receptors by glucocorticoids: functional and pathological significance. *Trends Pharmacol Sci* (1998) 19:317–21. doi:10.1016/S0165-6147(98)01229-2
24. Auphan N, DiDonato JA, Rosette C, Helmberg A, Karin M. Immunosuppression by glucocorticoids: inhibition of NFκB activity through induction of IκB synthesis. *Science* (1995) 270:286–90. doi:10.1126/science.270.5234.286
25. Scheinman RI, Cogswell PC, Lofquist AK, Baldwin AS Jr. Role of transcriptional activation of IκB in mediation of immunosuppression by glucocorticoids. *Science* (1995) 270:283–6. doi:10.1126/science.270.5234.283
26. Pandolfi J, Baz P, Fernández P, Discianni Lupi A, Payaslián F, Billordo LA, et al. Regulatory and effector T-cells are differentially modulated by dexamethasone. *Clin Immunol* (2013) 149:400–10. doi:10.1016/j.clim.2013.09.008
27. Cruz-Topete D, Cidlowski JA. One hormone, two actions: anti- and pro-inflammatory effects of glucocorticoids. *Neuroimmunomodulation* (2015) 22:20–32. doi:10.1159/000362724
28. Padgett DA, Loria RM. Endocrine regulation of murine macrophage function: effects of dehydroepiandrosterone, androstenediol, and androstenetriol. *J Neuroimmunol* (1998) 84:61–8. doi:10.1016/S0165-5728(97)00244-0
29. Kimura M, Tanaka S, Yamada Y, Kiuchi Y, Yamakawa T, Sekihara H. Dehydroepiandrosterone decreases serum tumor necrosis factor-alpha and restores insulin sensitivity: independent effect from secondary weight reduction in genetically obese Zucker fatty rats. *Endocrinology* (1998) 139:3249–53. doi:10.1210/endo.139.7.6118
30. del Rey A, Mahuad CV, Bozza VV, Bogue C, Farroni MA, Bay ML, et al. Endocrine and cytokine responses in humans with pulmonary tuberculosis. *Brain Behav Immun* (2007) 21:171–9. doi:10.1016/j.bbi.2006.06.005

31. Keven K, Uysal AR, Erdogan G. Adrenal function during tuberculous infection and effects of antituberculosis treatment on endogenous and exogenous steroids. *Int J Tuberc Lung Dis* (1998) 2:419–24.
32. Ellis E, Tayoub F. Adrenal function in tuberculosis. *Br J Dis Chest* (1986) 80:7–12. doi:10.1016/0007-0971(86)90003-3
33. Kelestimur F, Goktas Z, Gulmez I, Unluhizarci K, Bayram F, Ozesmi M, et al. Low dose (1 microg) adrenocorticotropin stimulation test in the evaluation of hypothalamo-pituitary-adrenal axis in patients with active pulmonary tuberculosis. *J Endocrinol Invest* (2000) 23:235–9. doi:10.1007/BF03343714
34. Mahuad C, Bay ML, Farroni MA, Bozza V, Del Rey A, Besedovsky H, et al. Cortisol and dehydroepiandrosterone affect the response of peripheral blood mononuclear cells to mycobacterial antigens during tuberculosis. *Scand J Immunol* (2004) 60:639–46. doi:10.1111/j.0300-9475.2004.01514.x
35. Angerami M, Suarez G, Quiroga MF, Pascutti MF, Salomon H, Bottasso O. Modulation of the phenotype and function of *Mycobacterium tuberculosis*-stimulated dendritic cells by adrenal steroids. *Int Immunol* (2013) 25:405–11. doi:10.1093/intimm/dxt004
36. Bongiovanni B, Mata-Espinosa D, D'Attilio L, Leon-Contreras JC, Marquez-Velasco R, Bottasso O, et al. Effect of cortisol and/or DHEA on THP1-derived macrophages infected with *Mycobacterium tuberculosis*. *Tuberculosis* (2015) 95:562–9. doi:10.1016/j.tube.2015.05.011
37. D'Attilio L, Bozza VV, Santucci N, Bongiovanni B, Dídoli G, Radcliffe S, et al. Inhibition of DHEA production by factors released from *M. tuberculosis*-stimulated peripheral blood mononuclear cells is abrogated by transforming growth factor-β neutralization. *Ann N Y Acad Sci* (2012) 1262:1–9. doi:10.1111/j.1749-6632.2012.06644.x
38. Mahuad C, Bozza V, Pezzotto SM, Bay ML, Besedovsky H, del Rey A, et al. Impaired immune responses in tuberculosis patients are related to weight loss that coexists with an immunoendocrine imbalance. *Neuroimmunomodulation* (2007) 14:193–9. doi:10.1159/000110646
39. van Lettow M, van der Meer JWM, West CE, van Crevel R, Semba RD. Interleukin-6 and human immunodeficiency virus load, nut not plasma leptin concentration, predict anorexia and wasting in adults with pulmonary tuberculosis in Malawi. *J Clin Endocrinol Metab* (2005) 90:4771–6. doi:10.1210/jc.2004-2539
40. Dallman M, Strack AM, Akana SF, Bradbury MJ, Hanson ES, Scribner KA, et al. Feast and famine: critical role of glucocorticoids with insulin in daily energy flow. *Front Neuroendocrinol* (1993) 14:303–47. doi:10.1006/frne.1993.1010
41. Kellendonk C, Eiden S, Kretz O, Schütz G, Schmidt I, Tronche F, et al. Inactivation of the GR in the nervous system affects energy accumulation. *Endocrinology* (2002) 143:2333–40. doi:10.1210/endo.143.6.8853
42. Plata-Salaman CR. Central nervous system mechanisms contributing to the cachexia-anorexia syndrome. *Nutrition* (2000) 16:1009–12. doi:10.1016/S0899-9007(00)00413-5
43. Saini A, Nassar A, Stewart CEH. Waste management – cytokines, growth factors and cachexia. *Cytokine Growth Factor Rev* (2006) 17:475–86. doi:10.1016/j.cytogfr.2006.09.006
44. Buchanan JB, Johnson RW. Regulation of food intake by inflammatory cytokines in the brain. *Neuroendocrinology* (2007) 86:183–90. doi:10.1159/000108280
45. Santucci N, D'Attilio L, Kovalevski L, Bozza V, Besedovsky H, del Rey A, et al. A multifaceted analysis of immune-endocrine-metabolic alterations in patients with pulmonary tuberculosis. *PLoS One* (2011) 6(10):e26363. doi:10.1371/journal.pone.0026363
46. Baumann H, Gauldie J. The acute phase response. *Immunol Today* (1994) 15:74–80. doi:10.1016/0167-5699(94)90137-6
47. Bonneaud C, Mazuc J, Gonzalez G, Haussy C, Chastel O, Faivre B, et al. Assessing the cost of mounting an immune response. *Am Nat* (2003) 203(161):367–79. doi:10.1086/346134
48. Hotamisligil G, Erbay E. The acute phase response. *Nat Rev Immunol* (2008) 8:923–34. doi:10.1038/nri2449
49. Phillips RS, Enwonwu CO, Okolo S, Hassan A. Metabolic effects of acute measles in chronically malnourished Nigerian children. *J Nutr Biochem* (2004) 15:281–8. doi:10.1016/j.jnutbio.2003.11.006
50. Woodward B. Protein, calories, and immune defenses. *Nutr Rev* (1998) 56:S84–92. doi:10.1111/j.1753-4887.1998.tb01649.x
51. Jacobson L. Hypothalamic-pituitary adrenocortical axis regulation. *Endocrinol Metab Clin North Am* (2005) 34:271–92. doi:10.1016/j.ecl.2005.01.003
52. Miyaura H, Iwata M. Direct and indirect inhibition of Th1 development by progesterone and glucocorticoids. *J Immunol* (2002) 168:1087–94. doi:10.4049/jimmunol.168.3.1087
53. Webster JI, Tonelli L, Sternberg EM. Neuroendocrine regulation of immunity. *Annu Rev Immunol* (2002) 20:125–63. doi:10.1146/annurev.immunol.20.082401.104914
54. La Cava A, Matarese G. The weight of leptin in immunity. *Nat Rev Immunol* (2004) 4(5):371–9. doi:10.1038/nri1350
55. Matarese G, Moschos S, Mantzoros CS. Leptin in immunology. *J Immunol* (2005) 174:3137–42. doi:10.4049/jimmunol.174.6.3137
56. Howard JK, Lord G, Matarese G, Vendetti S, Ghatei M, Ritter MA, et al. Leptin protects mice from starvation-induced lymphoid atrophy and increases thymic cellularity in ob/ob mice. *J Clin Invest* (1999) 104:1051–9. doi:10.1172/JCI6762
57. Lynch HE, Goldberg GL, Chidgey A, Van den Brink MR, Boyd R, Sempowski GD. Thymic involution and immune reconstitution. *Trends Immunol* (2009) 30(7):366–73. doi:10.1016/j.it.2009.04.003
58. Nobrega C, Cardona PJ, Roque S, Pinto do OP, Appelberg R, Correia-Neves M. The thymus as a target for mycobacterial infections. *Microbes Infect* (2007) 9:1521–9. doi:10.1016/j.micinf.2007.08.006
59. Savino W. The thymus is a common target organ in infectious diseases. *PLoS Pathog* (2006) 2:e62. doi:10.1371/journal.ppat.0020062
60. De Meis J, Savino W. Mature peripheral T cells are important to preserve thymus function and selection of thymocytes during *Mycobacterium tuberculosis* infection. *Immunotherapy* (2013) 5:573–6. doi:10.2217/imt.13.41
61. Nunes-Alves C, Nobrega C, Behar SM, Correia-Neves M. Tolerance has its limits: how the thymus copes with infection. *Trends Immunol* (2013) 34:502–10. doi:10.1016/j.it.2013.06.004
62. Borges M, Barreira-Silva P, Jordán M, Flórido M, Correia-Neves M, Appelberg R. Molecular and cellular mechanisms of *Mycobacterium avium*-induced thymic atrophy. *J Immunol* (2012) 189:3600–8. doi:10.4049/jimmunol.1201525
63. Nobrega C, Roque S, Nunes-Alves C, Coelhos A, Medeiros I, Castro AG. Dissemination of mycobacteria to the thymus renders newly generated T cells tolerant to the invading pathogen. *J Immunol* (2010) 184:351–8. doi:10.4049/jimmunol.0902152
64. Nobrega C, Nunes-Alves C, Cerqueira-Rodrigues B, Roque S, Barreira-Silva P, Behar SM, et al. T cells home to the thymus and control infection. *J Immunol* (2013) 190:1646–58. doi:10.4049/jimmunol.1202412
65. Hühns M, Erbersdobler A, Obliers A, Röpenack P. Identification of HPV types and *Mycobacterium tuberculosis* complex in historical long-term preserved formalin fixed tissues in different human organs. *PLoS One* (2017) 12:e0170353. doi:10.1371/journal.pone.0170353
66. Ruangnapa K, Anuntaseree W, Suntornlohanakul S. Tuberculosis of the thymus in a 6-month old infant with literature review. *Pediatr Infect Dis J* (2014) 33:210–2. doi:10.1097/INF.0000000000000016
67. Saieg MT, Bernardi Fdel C, Gonçalves R, Botter M, Saad Junior R, Pozzan G. Tuberculosis of the thymus. *J Bras Pneumol* (2007) 33:355–7. doi:10.1590/S1806-37132007000300020
68. Klein L, Kyewski B, Allen PM, Hogquist KA. Positive and negative selection of the T cell repertoire: what thymocytes see (and don't see). *Nat Rev Immunol* (2014) 14:377–91. doi:10.1038/nri3667
69. Liston A, Gray DH. Homeostatic control of regulatory T cell diversity. *Nat Rev Immunol* (2014) 14:154–65. doi:10.1038/nri3605
70. Savino W, Mendes-da-Cruz DA, Lepletier A, Dardenne M. Hormonal control of T-cell development in health and disease. *Nat Rev Endocrinol* (2016) 12:77–89. doi:10.1038/nrendo.2015.168
71. Dixit VD. Thymic fatness and approaches to enhance thymopoietic fitness in aging. *Curr Opin Immunol* (2010) 22:521–8. doi:10.1016/j.coi.2010.06.010
72. Aspinall R, Andrew D. Thymic involution in aging. *J Clin Immunol* (2000) 20:250–6. doi:10.1023/A:1006611518223
73. Hakim FT, Memon SA, Cepeda R, Jones EC, Chow CK, Kasten-Sportes C, et al. Age-dependent incidence, time course, and consequences of thymic renewal in adults. *J Clin Invest* (2005) 115:930–9. doi:10.1172/JCI200522492

74. Castermans E, Hannon M, Dutrieux J, Humblet-Baron S, Seidel L, Cheynier R, et al. Thymic recovery after allogeneic hematopoietic cell transplantation with non-myeloablative conditioning is limited to patients younger than 60 years of age. *Haematologica* (2011) 96:298–306. doi:10.3324/haematol.2010.029702
75. Jondal M, Pazirandeh A, Okret S. Different roles for glucocorticoids in thymocyte homeostasis? *Trends Immunol* (2004) 25:595–600. doi:10.1016/j.it.2004.09.003
76. Talaber G, Jondal M, Okret S. Local glucocorticoid production in the thymus. *Steroids* (2015) 103:58–63. doi:10.1016/j.steroids.2015.06.010
77. Pazirandeh A, Jondal M, Okret S. Conditional expression of a glucocorticoid receptor transgene in thymocytes reveals a role for thymic-derived glucocorticoids in thymopoiesis*in vivo*. *Endocrinology* (2005) 146:2501–7. doi:10.1210/en.2004-0943
78. Qiao S, Okret S, Jondal M. Thymocyte-synthesized glucocorticoids play a role in thymocyte homeostasis and are down-regulated by adrenocorticotropic hormone. *Endocrinology* (2009) 150:4163–9. doi:10.1210/en.2009-0195
79. Savino W, Dardenne M. Nutritional imbalances and infections affect the thymus: consequences on T-cell-mediated immune responses. *Proc Nutr Soc* (2010) 69:636–43. doi:10.1017/S0029665110002545
80. Savino W, Dardenne M. Neuroendocrine control of thymus physiology. *Endocr Rev* (2000) 21:412–43. doi:10.1210/edrv.21.4.0402
81. Mittelstadt PR, Monteiro JP, Ashwell JD. Thymocyte responsiveness to endogenous glucocorticoids is required for immunological fitness. *J Clin Invest* (2012) 122:2384–94. doi:10.1172/JCI63067
82. Pálinkás L, Talabér G, Boldizsár F, Bartis D, Németh P, Berki T. Developmental shift in TcR-mediated rescue of thymocytes from glucocorticoid-induced apoptosis. *Immunobiology* (2008) 213:39–50. doi:10.1016/j.imbio.2007.06.004
83. Díaz A, Bongiovanni B, D'Attilio L, Santucci N, Dídoli G, Fernández R, et al. The clinical recovery of tuberculosis patients undergoing specific treatment is associated with changes in the immune and neuroendocrine responses. *Pathog Dis* (2017) 75(7). doi:10.1093/femspd/ftx087
84. Greenwtood PL, Bell AW. Consequences of intra-uterine growth retardation for postnatal growth, metabolism and thymic pathophysiology. *Reprod Suppl* (2003) 61:195–206.
85. Gruver AL, Ventevogel MS, Sempowski GD. Leptin receptor is expressed in thymus medulla and leptin protects against thymic remodeling during endotoxemia-induced thymus involution. *J Endocrinol* (2009) 203:75–85. doi:10.1677/JOE-09-0179
86. May M, Holmes E, Rogers W, Poth M. Protection from glucocorticoid induced thymic involution by dehydroepiandrosterone. *Life Sci* (1990) 46:1627–31. doi:10.1016/0024-3205(90)90394-7
87. Chmielewski V, Drupt F, Morfin R. Dexamethasone-induced apoptosis of mouse thymocytes: prevention by native 7alpha-hydroxysteroids. *Immunol Cell Biol* (2000) 78:238–46. doi:10.1046/j.1440-1711.2000.00905.x
88. Blauer KL, Poth M, Rogers WM, Bernton EW. Dehydroepiandrosterone antagonizes the suppressive effects of dexamethasone on lymphocyte proliferation. *Endocrinology* (1991) 129:3174–9. doi:10.1210/endo-129-6-3174
89. Liang J, Yao G, Yang L, Hou Y. Dehydroepiandrosterone induces apoptosis of thymocyte through Fas/Fas-L pathway. *Int Immunopharmacol* (2004) 4:1467–75. doi:10.1016/j.intimp.2004.06.010
90. Yan CH, Jiang XF, Pei X, Dai YR. The *in vitro* antiapoptotic effect of dehydroepiandrosterone sulfate in mouse thymocytes and its relation to caspase-3/caspase-6. *Cell Mol Life Sci* (1999) 56:543–7. doi:10.1007/s000180050451
91. Hu Y, Cardounel A, Gursoy E, Anderson P, Kalimi M. Anti-stress effects of dehydroepiandrosterone: protection of rats against repeated immobilization stress-induced weight loss, glucocorticoid receptor production, and lipid peroxidation. *Biochem Pharmacol* (2000) 59:753–62. doi:10.1016/S0006-2952(99)00385-8
92. Filipin MV, Caetano LC, Brazão V, Santello FH, Toldo MP, do Prado JC Jr. DHEA and testosterone therapies in *Trypanosoma cruzi*-infected rats are associated with thymic changes. *Res Vet Sci* (2010) 89:98–103. doi:10.1016/j.rvsc.2010.01.016
93. de Mello-Coelho V, Gagnerault MC, Souberbielle JC, Strasburger CJ, Savino W, Dardenne M, et al. Growth hormone and its receptor are expressed in human thymic cells. *Endocrinology* (1998) 139:3837–42. doi:10.1210/endo.139.9.6199
94. Smaniotto S, Mendes-da-Cruz DA, Carvalho-Pinto CE, Araujo LM, Dardenne M, Savino W. Combined role of extracellular matrix and chemokines on peripheral lymphocyte migration in growth hormone transgenic mice. *Brain Behav Immun* (2010) 24:451–61. doi:10.1016/j.bbi.2009.11.014
95. Smaniotto S, de Mello-Coelho V, Villa-Verde DM, Pléau JM, Postel-Vinay MC, Dardenne M, et al. Growth hormone modulates thymocyte development *in* vivo through a combined action of laminin and CXC chemokine ligand 12. *Endocrinology* (2005) 146:3005–17. doi:10.1210/en.2004-0709
96. Carreno PC, Sacedon R, Jimenez E, Vicente A, Zapata AG. Prolactin affects both survival and differentiation of T-cell progenitors. *J Neuroimmunol* (2005) 160:135–45. doi:10.1016/j.jneuroim.2004.11.008
97. Kelley KW, Brief S, Westly HJ, Novakofski J, Bechtel PJ, Simon J, et al. GH3 pituitary adenoma cells can reverse thymic aging in rats. *Proc Natl Acad Sci U S A* (1986) 83:5663–7. doi:10.1073/pnas.83.15.5663
98. Villa-Verde DM, de Mello-Coelho V, Farias-de-Oliveira DA, Dardenne M, Savino W. Pleiotropic influence of triiodothyronine on thymus physiology. *Endocrinology* (1993) 133:867–75. doi:10.1210/endo.133.2.8344222
99. Chen YK, Yeh CL, Chen YL, Wang SC, Cheng RH, Kao PF. The frequency and spectrum of thymus 2-[fluorine-18] fluoro-2-deoxy-D-glucose uptake patterns in hyperthyroidism patients. *Acad Radiol* (2011) 18:1292–7. doi:10.1016/j.acra.2011.05.011
100. Yellayi S, Teuscher C, Woods JA, Welsh TH Jr, Tung KS, Nakai M, et al. Normal development of thymus in male and female mice requires estrogen/estrogen receptor-αsignaling pathway. *Endocrine* (2000) 12:207–13. doi:10.1385/ENDO:12:3:207
101. Barr IG, Khalid BA, Pearce P, Toh BH, Bartlett PF, Scollay RG, et al. Dihydrotestosterone and estradiol deplete corticosensitive thymocytes lacking in receptors for these hormones. *J Immunol* (1982) 128:2825–8.
102. Bauer M, Wieck A, Petersen LE, Baptista TSA. Neuroendocrine and viral correlates of premature immunosenescence. *Ann N Y Acad Sci* (2015) 1351:11–21. doi:10.1111/nyas.12786
103. Bauer ME, Jeckel CM, Luz C. The role of stress factors during aging of the immune system. *Ann N Y Acad Sci* (2009) 1153:139–52. doi:10.1111/j.1749-6632.2008.03966.x
104. Salama R, Sadaie M, Hoare M, Narita M. Cellular senescence and its effector programs. *Genes Dev* (2014) 28:99–114. doi:10.1101/gad.235184.113
105. Correia-Melo C, Hewitt G, Passos JF. Telomeres, oxidative stress and inflammatory factors: partners in cellular senescence? *Longev Healthspan* (2014) 3:1. doi:10.1186/2046-2395-3-1
106. Monti D, Ostan R, Borelli V, Castellan G, Franceschi C. Inflammaging and human longevity in the omics era. *Mech Ageing Dev* (2016) 165:129–38. doi:10.1016/j.mad.2016.12.008
107. Morrisette-Thomas V, Cohen AA, Fülöp T, Riesco É, Legault V, Li Q, et al. Inflammaging does not simply reflect increases in pro-inflammatory markers. *Mech Ageing Dev* (2014) 139:49–57. doi:10.1016/j.mad.2014.06.005
108. Shaw AC, Goldstein DR, Montgomery RR. Age-dependent dysregulation of innate immunity. *Nat Rev Immunol* (2013) 13:875–87. doi:10.1038/nri3547
109. Mahbub S, Brubaker AL, Kovacs EJ. Aging of the innate immune system: an update. *Curr Immunol Rev* (2011) 7:104–15. doi:10.2174/157339511794474181
110. Arnold CR, Wolf J, Brunner S, Herndler-Brandstetter D, Grubeck-Loebenstein B. Gain and loss of T cell subsets in old age – age-related reshaping of the T cell repertoire. *J Clin Immunol* (2011) 31:137–46. doi:10.1007/s10875-010-9499-x

Conflict of Interest Statement: Authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Endocrinology

REVIEW
published: 14 June 2018
doi: 10.3389/fendo.2018.00334

Role of Hormonal Circuitry Upon T Cell Development in Chagas Disease: Possible Implications on T Cell Dysfunctions

Ana Rosa Pérez[1], Alexandre Morrot[2,3], Vinicius Frias Carvalho[4,5], Juliana de Meis[5,6] and Wilson Savino[5,6]*

[1] Institute of Clinical and Experimental Immunology (IDICER-CONICET UNR), Rosario, Argentina, [2] Faculty of Medicine, Tuberculosis Research Center, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil, [3] Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, [4] Laboratory of Inflammation, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, [5] Laboratory on Thymus Research, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, [6] National Institute of Science and Technology on Neuroimmunomodulation (INCT-NIM), Rio de Janeiro, Brazil

OPEN ACCESS

Edited by:
Vincent Geenen, University of Liège, Belgium

Reviewed by:
Arturo Ortega, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), Mexico
Susana Elba Gea, National University of Cordoba, Argentina

***Correspondence:**
Ana Rosa Pérez
perez@idicer-conicet.gob.ar,
perez_anarosa@yahoo.com.ar

Specialty section:
This article was submitted to Neuroendocrine Science, a section of the journal Frontiers in Endocrinology

Received: *27 November 2017*
Accepted: *31 May 2018*
Published: *14 June 2018*

Citation:
Pérez AR, Morrot A, Carvalho VF, de Meis J and Savino W (2018) Role of Hormonal Circuitry Upon T Cell Development in Chagas Disease: Possible Implications on T Cell Dysfunctions. Front. Endocrinol. 9:334. doi: 10.3389/fendo.2018.00334

T cell response plays an essential role in the host resistance to infection by the protozoan parasite *Trypanosoma cruzi*, the causative agent of Chagas disease. This infection is often associated with multiple manifestations of T cell dysfunction, both during the acute and the chronic phases of disease. Additionally, the normal development of T cells is affected. As seen in animal models of Chagas disease, there is a strong thymic atrophy due to massive death of $CD4^+CD8^+$ double-positive cells by apoptosis and an abnormal escape of immature and potentially autoreactive thymocytes from the organ. Furthermore, an increase in the release of corticosterone triggered by *T. cruzi*-driven systemic inflammation is strongly associated with the alterations seen in the thymus of infected animals. Moreover, changes in the levels of other hormones, including growth hormone, prolactin, and testosterone are also able to contribute to the disruption of thymic homeostasis secondary to *T. cruzi* infection. In this review, we discuss the role of hormonal circuits involved in the normal T cell development and trafficking, as well as their role on the thymic alterations likely related to the peripheral T cell disturbances largely reported in both chagasic patients and animal models of Chagas disease.

Keywords: Chagas disease, thymus atrophy, thymocytes, hypothalamus–pituitary–adrenal axis, growth hormone, prolactin

INTRODUCTION

Chagas disease, or American trypanosomiasis, is a tropical neglected illness caused by the hemoflagellate protozoan *Trypanosoma cruzi*. Chagas disease transmission to humans can be classified in primary (vectorial, blood transfusion, congenital, and orally) and secondary (less frequent, such as laboratory accident, handling of infected animals, organ transplantation from infected donors, and hypothetically through sexual) routes of *T. cruzi* infection (1, 2). Presently, oral transmission of human Chagas disease is the most important transmission route in the Brazilian Amazon region, mainly secondary to food/beverage contamination with *T. cruzi*. It is noteworthy that oral transmission has been associated with high mortality and morbidity, increased prevalence and severity of the cardiac pathology (myocarditis) (3–6). Argentina, Bolivia, Colombia, Ecuador, French Guiana, and Venezuela have also reported acute Chagas disease cases associated with contaminated food consumption (7–9).

Trypanosoma cruzi infection is presently considered as a worldwide health problem with deficiencies in treatment, absence of appropriated vaccines and world spreading (10, 11). The infection leads to an acute phase, with symptoms such as fever, muscle pain, swollen lymph nodes, hepatosplenomegaly, edema, tachycardia, dyspnea, pericardial effusion and inflammatory reaction at the vector's biting site of the vector (chagoma) (2, 12). During the acute phase, circulating parasite numbers are systemically increased, and they are able to infect several tissues and cell types, such as skeletal and cardiac myocytes, macrophages, fibroblasts, neurons and epithelial cells. For this reason, amastigote nests were already described in glands, skeletal muscle, as well as, lymphoid and nervous tissues (11, 13). Following recovery from the acute phase, the patient enters into a long indeterminate, latent, phase with no symptoms and very low parasitism. The latent infection may remain silent for 10–30 years. One-third of infected patients in the latent phase develop clinical symptoms as chronic cardiac dysfunction (cardiomyopathy), megacolon or megaesophagus. It is believed that chronic chagasic cardiomyopathy has an autoimmune pathophysiological component, with involvement of T and B autoreactive cells, as well as promoted by the persistence of the parasite. At this phase, life expectancy decreases about 9 years in these clinical forms of chronic patients (14).

T Cell Changes During *T. cruzi* Infection

In the immune system, *T. cruzi* infection promotes changes in the dynamics and in the size of T lymphocyte populations, contributing to regional response in primary, including thymus and secondary lymphoid organs (15). In infected mice, the thymus suffers a strong atrophy in the acute phase, due to massive death of $CD4^+CD8^+$ double-positive (DP) and $CD4^+Foxp3^+$ regulatory T cells (Treg) by apoptosis, accompanied by an abnormal escape of immature and potentially autoreactive T lymphocytes from the organ (11, 16). Interestingly, T cell abnormal escape was also documented in chronically *T. cruzi*-infected patients (17, 18). On the other hand, it is known that under physiological conditions, the re-entry of $CD4^+$ and $CD8^+$ T cells into the thymus is restricted to activated/memory cells (19), being driven by CCL2/CCR2 interactions (20). Some authors speculate that the re-entrance of T effector cells may influence the tolerance induction by promoting Treg development, since they represent the main source of IL-2 (21). Furthermore, Treg with a clear maturational phenotype were observed in the infected thymus, suggesting that they may correspond to peripheral Treg that have re-entered into the thymus (16). In any case, the physiological consequences of the Treg cell re-entry into the thymus remains undetermined.

Diverse groups have shown an expansion in secondary lymphoid organs such spleen and subcutaneous lymph nodes due to T and B cell polyclonal activation. In contrast, the mesenteric lymph nodes and Payer's patches show atrophy and T lymphocyte death (15, 22–33).

An increase in IL-2 production is involved in subcutaneous lymph nodes hyperplasia in *T. cruzi* infection (15, 31). Spleen and subcutaneous lymph node hypertrophy is a consequence of tissue T/B lymphocyte activation and proliferation (15, 23, 31, 34, 35). Moreover, trans-sialidase, racemase, and *T. cruzi* DNA seem to contribute to T and/or B lymphocyte activation and cytokine production by interfering with interaction between dendritic cells and lymphocytes (36–40). In contrast to the hyperplasia seen in spleen and subcutaneous lymph nodes of infected mice, mesenteric lymph node atrophy is related to a local decrease in IL-2 and IL-4 production, with apoptotic death of T/B lymphocytes (15). In a second vein, it has been shown in the mouse model that splenectomy or mesenteric lymph node excision prior to *T. cruzi* inoculum increases susceptibility to infection, suggesting that these lymphocytes are involved in *T. cruzi* host immune response (15, 22–33).

SYSTEMIC HORMONAL IMBALANCE IN CHAGAS DISEASE

Endocrine and immune systems control several physiological, biochemical, and functional activities in the organism both during homeostasis, including early development and aging (41), as in pathological situations, such as infectious and metabolic diseases (42, 43). Immunoendocrine interactions occur through bidirectional circuits, characterized by highly specialized signaling molecules known as cytokines and hormones, respectively (44). Given the extensive diversity of interactions between endocrine and immune cells, it is conceivable that disturbances of one or more of these components of the immunoendocrine axes lead to the development and/or exacerbation of several illnesses, including Chagas disease (42).

The hormonal imbalance in patients with Chagas disease has been discussed since the discovery and description by Carlos Chagas, who divided the symptomatology of chronic form of American Trypanosomiasis according to thyroid, heart, and central nervous system disease. In fact, the inclusion of the thyroid form of the disease was based on both clinical aspects, association of goiter with myxedema, and the detection of the parasite and inflammation in thyroid during autopsy (45). Currently, it is believed that Chagas disease by itself is not able to cause goiter, but may predispose patients to develop goiter (46).

One of the main endocrine circuits studied in Chagas disease is the hypothalamus–pituitary–adrenal (HPA) axis, since the release of glucocorticoid (GC) hormones is a protective mechanism of the host against the harmful effects of pro-inflammatory cytokines (47). Acute *T. cruzi* infection induces increased corticosterone levels in both C57BL/6 and BALB/c mouse strains (48), indicating a hyperactivation of HPA axis. Such an increase in circulating corticosterone levels is in close correlation with the hypertrophy of adrenal glands, including its *zona fasciculata*, and a rise in the expression of several steroidogenic enzymes, such as cytochrome P450, family 11, subfamily A, polypeptide 1 (CYP11A1), CYP11B1, 11β-hydroxysteroid dehydrogenase type 1 (HSD1), and steroidogenic acute regulatory protein (StAR) (49).

This HPA axis activation observed in experimental models of Chagas disease is associated with the presence of nests of *T. cruzi* amastigotes in the adrenals, as well as parasite-derived antigens in both adrenals and pituitary gland (50). Although by now, the underlyning mechanisms are not fully elucidated, the presence of *T. cruzi*-derived antigens (proteins, DNA, or glycolipids) in the endocrine glands of HPA axis may promote a local inflammatory response *via* the engagement of TLRs, as

shown in bacterial models of infection (51). In particular, the stimulation of TLR-9, which recognizes *T. cruzi* DNA (52), may cause the local production of cytokines and consequent increase in the release of corticosterone, as seen in a model of sepsis (53). Similarly, TLR-2 or TLR-4 pathways might be stimulated by TLR agonists expressed by *T. cruzi* like GPI or GIPL anchors, respectively (54). In fact, C57BL/6 mice infected with *T. cruzi* showed, not only in plasma but also intra-adrenal, increased levels of TNF-α, IL-1β, and IL-6 (55), suggesting that these proinflammatory cytokines are involved in the hyperactivation of HPA axis at different levels.

Although infected mice presented the parasite and a pronounced inflammatory response in the pituitary gland, the systemic levels of adrenocorticotropic hormone (ACTH) are not changed (49, 50), suggesting that the increase in circulating corticosterone levels noted in infected mice occurs independently of ACTH. In fact, both systemic and intra-adrenal cytokine production may favor adrenal inflammation during infection, which can directly trigger and sustain an alternative way of adrenal secretion of GC, resulting uncoupled from the hypothalamic–pituitary unit (56). Structural alterations like vascular changes within the endocrine microenvironment may also lead to a transient HPA dysfunction (56). Also, local inflammation driven by the presence of *T. cruzi* or their antigens may promote the income of inflammatory cells. Strikingly, adrenals of infected mice showed leukocyte infiltration, characterized by the presence of $CD8^+$ and $CD4^+$ T lymphocytes, as well as macrophages and enhanced expression of extracellular matrix (ECM) deposition, including fibronectin and laminin (44). These ECM molecules might fix pathogen-derived antigens as well as pro-inflammatory cytokines released during immune response, thus contributing to the establishment of inflammation and sustaining GC production (56).

Pituitary hormones, including growth hormone (GH) and prolactin (PRL), act as modulators of the immune system (57, 58). Similarly to GC, GH and PRL are considered stress-related hormones (59, 60), having opposing actions of GC on the viability and proliferation of thymic cells (61). In GH-/PRL-secreting GH3 cells, the infection with *T. cruzi in vitro* induces a reduction in the secretion of both GH and PRL (62). These results suggest that *T. cruzi* infection decreases GH and PRL production by the pituitary. In fact, chagasic patients showed decreased GH levels in response to glucose or insulin compared to healthy subjects (63), and mice infected with *T. cruzi* presented a reduction in plasma PRL levels (64). In effect, the low production of PRL by pituitary induced by *T. cruzi* infection seems to directly affect the high corticosterone synthesis by the adrenals (65). Interestingly, while asymptomatic patients showed a tendency to diminish the secretion of GH, individuals with severe cardiomyopathy show increased levels of this hormone and also an altered GH/IGF-1, suggesting an imbalance in this axis (65).

Besides GC and pituitary hormones, some gonadal steroid hormones, including dehydroepiandrosterone-sulfate (DHEA-s) and testosterone, can be altered in human or experimental Chagas disease (66, 67). Animals infected with *T. cruzi* presented a reduction in serum testosterone levels in the acute phase of infection. However, histological analyses in testes, seminal vesicles, and epididymis did not reveal any differences between control and infected animals (68). In addition, *T. cruzi*-infected animals showed an increase in circulating levels of estradiol (67). Regarding DHEA-s levels, rats infected with *T. cruzi* did not alter the DHEA-s systemic levels. However, chronic chagasic patients with different degrees of myocarditis presented a marked reduction in DHEA-s levels. Interestingly, although the alterations in the levels of DHEA in animals are not seen in patients with Chagas disease, both animals and patients presented an increase in GC/DHEA-s ratio, which is important for the development of an anti-inflammatory milieu (66, 67).

HORMONES AND THEIR RELATIONSHIP WITH THYMUS ATROPHY IN *T. cruzi* INFECTION

T cell response plays an essential role in the host resistance to the *T. cruzi* infection, but sub-clinic and clinic manifestations of Chagas disease can be associated with multiple manifestations of T cell dysfunction (69–73). Additionally, as seen in animal models of Chagas disease, there is a strong thymic atrophy characterized by loss of thymus weight, massive death of $CD4^+CD8^+$ DP cells by caspase-dependent apoptosis (32), alterations in the double-negative (DN) T-cell population (74, 75), depletion of thymic Treg (16) and also an abnormal and premature escape of immature and potentially autoreactive DP and DN thymocytes from the organ (17, 26, 74, 76). Furthermore, it has been recently described that during experimental *T. cruzi* infection, bone marrow aplasia and a diminution in common lymphoid progenitors appear before thymic alterations (75).

Due to the possible autoimmune component of chagasic myocarditis, it is plausible to hypothesize that thymic selection mechanisms could be altered as a consequence of the infection. In this regard, in BALB/c mice, some T-cell receptor (TCR) Vβ families, which under normal conditions should have undergone negative selection through apoptosis, appear in the periphery of the immune system during *T. cruzi* infection and might potentially conduce to autoimmune reactions (77). Nevertheless, in the same study, potentially autoimmune mature T cells were not seen within the thymus. Using an (OVA)-specific TCR transgenic system, we confirmed that the negative selection process is normal during experimental *T. cruzi* infection. In addition, the expression of autoimmune regulator factor (AIRE) expression and tissue-restricted antigen genes were normal in the thymus of infected animals (17). However, similarly to what is described in the murine model, activated DP T cells with an activated phenotype are found in the blood of patients with chronic Chagas disease in association with severe myocarditis (17), suggesting that some intrathymic checkpoints might be failed. This may have related to T cell trafficking alterations due to changes in the patterns the ECM protein deposition within the organ, expression of ECM receptors on thymocytes and thymic Tregs, as well as changes in cell migration-related cytokines (16, 32, 78, 79).

Normal T cell development is tightly controlled not only by cell–cell interactions and cytokines, but also by hormones, interacting *via* a diversity of endocrine and paracrine pathways, acting

on thymocytes and thymic microenvironmental cells *via* specific receptors (42, 80). Moreover, thymic cells not only respond to systemic levels of hormones but also constitutively synthetize and secrete hormones locally, such as GC, GH and PRL. In this context, disturbances in hormone levels caused by inflammation can interfere with the normal T cell development. Accordingly, increased evidence indicates that the thymic alterations seen during *T. cruzi* infection are strongly associated to hormonal imbalance, involving systemic or intrathymic axes.

The HPA Axis

It is well known that, if not controlled, systemic effects of GC on the adaptive immunity can promote immunological disturbances. The HPA axis activation, through the production and action of GC, plays a major role in protecting the host against the inflammatory acute stress caused by *T. cruzi* infection (48, 55). Nevertheless, immature DP thymocytes are major targets of HPA axis activation, since enhanced levels of GC seen in experimental acute *T. cruzi* infection induce DP thymocyte depletion through caspase-dependent apoptosis (32, 81). In this regard, blockade of GC receptor activity with RU486 prevented DP thymocyte apoptosis (48, 55) together with caspase-8 and caspase-9 activation (32). Interestingly, both thymic epithelial cells and DP thymocytes can also synthetize GC, suggesting that both paracrine and autocrine loops influence thymocyte survival during *T. cruzi* infection (82, 83). In addition, *T. cruzi* is able to infect thymic epithelial cells (84), indicating that the parasite *per se* may alter the local production of hormones and determining thymocyte fate. Yet, this hypothesis needs experimental confirmation.

GH and PRL

Prolactin is not only produced in the anterior pituitary gland but also in a range of tissues including adipose tissue, skin, and thymus. Actually, both GH and PRL exert relevant roles upon thymus physiology and are constitutively produced and secreted by thymocytes and thymic epithelial cells (TEC) (85–87). Increased intrathymic expression of GH leads to an enlarged thymus, as can be observed in transgenic mice that overexpress the hormone or in individuals treated with recombinant forms of GH (88–90). In addition, GH and IGF-1 (the hormone that mediates several GH effects) favor thymocyte migration, augmenting ECM deposition (85). Moreover, specific receptors for GH, IGF-1 and PRL are constitutively expressed by TEC and thymocytes, indicating autocrine and paracrine regulatory loops, in addition to the systemic effects of these hormones (57, 90).

The action of these anti-stress hormones is actually one of the ways that counterregulate systemically or in an organ-specific fashion, the action of the GC produced during *T. cruzi* infection. We have shown that PRL plays a critical role in balancing the effects of corticosterone in the thymus during *T. cruzi* infection (65, 74). In the mouse model of *T. cruzi* acute infection, we found an intrathymic cross-talk between GC receptors (GR) and PRL receptors that seems to work to counteract the effects of the infection, toward the neutralization of GC-related systemic deleterious effects on DP thymocyte survival during parasite-induced thymic atrophy. Furthermore, we showed that injection of metoclopramide (known to enhance PRL secretion by the pituitary gland), during experimental infection, preserved the thymus from atrophy during infection with *T. cruzi* (65). This event was associated with partial prevention of DP thymocyte apoptosis as well as thymic release of undifferentiated and potentially autoreactive DP cells to the peripheral lymphoid tissues. These findings point to a modulation of the stress-related hormonal circuits in the intrathymic T cell development during *T. cruzi* infection.

Testosterone and DHEA

Androgens in general, and especially testosterone, have immunosuppressive actions on the immune system, whereas the androgen DHEA seems to have immunostimulating properties, and counteracts the immunosuppressive effects of GC (91). In a second vein, it is widely accepted that sexual dimorphism is strongly related with differences in immune function and disease outcome. Concerning experimental Chagas disease, females are more resistant to infection than males, and androgen depletion improved the resistance against *T. cruzi* (92–94). Interestingly, in male mice, DP thymocyte death within thymic nurse cells seems to be caused by testosterone (95) and testosterone supplementation causes a diminution in thymocyte proliferation (96). Unlike GC, known to activate caspase-8 and caspase-9-mediated apoptosis in thymocytes, testosterone is able to activate thymocyte apoptosis through a caspase-3-dependent pathway (95). Studies in the rat model of *T. cruzi* acute infection revealed that DHEA supplementation promotes thymocyte proliferation, suggesting that DHEA treatment may prevent DP loss and other thymic alterations (96). Nevertheless, more studies are needed to evaluate the role of sex hormones in the thymic atrophy that occurs during *T. cruzi* infection.

METABOLIC ALTERATIONS AND ADIPOKINES

In parallel to the endocrine imbalance, animals infected with *T. cruzi* also show a clear metabolic disturbance, including hypoglycemia, weight loss and leptin alterations (97). It is known that, besides controlling saciety, leptin plays protective affects upon intrathymic T cell development under physiologic conditions (98, 99). Nevertheless, in acute *T. cruzi*-infected C57BL/6 mice, its systemic and adipose tissue derived-expression is sharply diminished, suggesting that its loss may fuel thymic atrophy (97) However, and unlike what happens in models of experimental endotoxemia (100), leptin replacement during the acute infection, while attenuating GC release, fails in reversing thymic atrophy (97). The reason of this difference should be investigated, but it is possible to speculate that thymic ObR expression during *T. cruzi* infection could be also diminished, as previously observed at the hypothalamic level (97). In this regard, when infected db/db mice (that lack ObR) were reconstituted with the brain ObR, the infection was less obvious (101). These data suggest that leptin axis is dysregulated during infection. Strikingly, in chronic obese model of infection and also in human chronic disease, it was reported that adipokine disturbances are related to myocardial damage and heart autonomic dysfunction (102, 103), while their effects upon T cell dynamics has not been estimated.

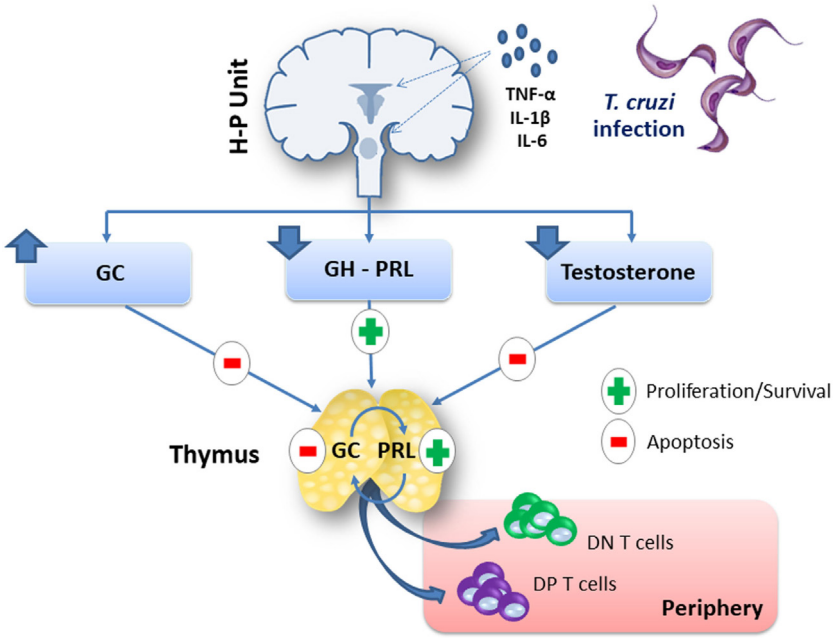

FIGURE 1 | Systemic and intrathymic hormonal imbalance affects the thymus during experimental *Trypanosoma cruzi* infection. Acute *T. cruzi* infection in mice induces a rise in plasma levels of proinflammatory cytokines, which are involved in the hyperactivation of the hypothalamus–pituitary–adrenal (HPA) axis. Pro-inflammatory cytokines can enhance HPA axis activation, by acting at the hypothalamus–pituitary unit and/or on peripheral glands, i.e., the adrenals. *In situ* inflammatory reactions caused by *T. cruzi*-derived antigens or structural changes like vascular alterations or an enhanced extracellular matrix deposition in the endocrine microenvironment may also lead to sustain glucocorticoid hormone (GC) levels. The increment of systemic and intrathymic GC levels causes thymic atrophy by depletion of $CD4^+CD8^+$ double-positive (DP) thymocytes through apoptosis. In parallel, there is an abnormal export of immature DP and double-negative (DN) T cells to the periphery of the immune system. Growth hormone (GH) and prolactin (PRL) have positive effects upon the thymus, but *T. cruzi* infection decreases GH and PRL production by pituitary cells. Male animals acutely infected with *T. cruzi* also present a reduction in serum testosterone levels, although DP thymocyte death seems to be induced by this androgen, whereas testosterone supplementation induced a diminution in thymocyte proliferation. Abbreviation: H–P unit, hypothalamus–pituitary unit.

TABLE 1 | Effects of hormonal imbalance upon thymocytes during *Trypanosoma cruzi* infection.

	GC	DHEA	PRL	GH	Testo	Leptin (*)	Reference
Weight/size	↓	↑	↑	↑	↓	↓	(18, 42, 44, 45, 53, 104)
Cellularity	↓	↑	↑	↑	↓	↓	(25, 42, 44, 46, 54, 55, 66, 73, 88, 104)
Apoptosis of DP cells	↑	↓	↓	↓	↑	↓	(25, 42, 44, 46, 54, 55, 73, 87, 88, 104)
Loss of Tregs	↑	ND	ND	ND	ND	ND	(66)
Vβ T-cell repertoire/negative selection	ND	ND	ND	ND	ND	ND	(19, 67)
Altering intrathymic cell migration	ND	ND	ND	ND	ND	ND	(21, 66, 69, 80)
Escape of DP/DN cells to periphery	ND	ND	↓	ND	ND	ND	(11, 55, 64, 67, 89, 105)

DP, $CD4^+CD8^+$ double-positive; GC, glucocorticoids; DHEA, dehidroepiandrosterone; PRL, prolactin; GH, growth hormone; Testo, testosterone; ND, not determined; ↑, increase; ↓, decrease; (*) effects caused by administration.

CONCLUSION

There is no doubt that acute *T. cruzi* infection induces an immunoendocrine imbalance, which somehow favors the ability of the parasite to settle in the host, and the development of distinct pathological events, among which the massive thymocyte death and consequent thymic atrophy. Yet, this is a complex network of events (summarized in **Figure 1** and **Table 1**) that needs further investigation, including the possibility of endocrine axes being target for complementary therapeutic intervention in Chagas disease.

AUTHOR CONTRIBUTIONS

All authors contribute equally to the manuscript: AP, AM, VC, JM, and WS.

FUNDING

This study was supported by Foncyt (PICT 2008-1892 and 2013-1892), PIP CONICET 0641, SecCyT-UNR 1MED244 and 1MED348, ASACTeI 2010-120-14 (Argentine) FAPERJ, CAPES, CNPq, FOCEM-Mercosur, Fiocruz, Inct-NIM (Brazil).

REFERENCES

1. Dorn P, Buekens P, Hanford E. Whac-a-mole: future trends in Chagas transmission and the importance of a global perspective on disease control. *Future Microbiol* (2007) 2:365–7. doi:10.2217/17460913.2.4.365
2. Tarleton RL, Reithinger R, Urbina JA, Kitron U, Gurtler RE. The challenges of Chagas disease – grim outlook or glimmer of hope. *PLoS Med* (2007) 4(12):e332. doi:10.1371/journal.pmed.0040332
3. Barbosa-Ferreira JM, Nobre AF, Maldonado JG, Borges-Pereira J, Zauza PL, Coura JR. [Stroke in a chronic autochthonous chagasic patient from the Brazilian Amazon]. *Rev Soc Bras Med Trop* (2010) 43(6):751–3. doi:10.1590/S0037-86822010000600034
4. Ianni BM, Mady C. [The sugarcane juice was delicious, but…]. *Arq Bras Cardiol* (2005) 85(6):379–81.
5. Vinas Albajar P, Laredo SV, Terrazas MB, Coura JR. [Dilated cardiomyopathy in patients with chronic chagasic infection: report of two fatal autochthonous cases from Rio Negro, State of Amazonas, Brazil]. *Rev Soc Bras Med Trop* (2003) 36(3):401–7. doi:10.1590/S0037-86822003000300013
6. Pinto AY, Harada GS, Valente V, Abud JE, Gomes F, Souza GC, et al. [Cardiac attacks in patients with acute Chagas disease in a family micro-outbreak, in Abaetetuba, Brazilian Amazon]. *Rev Soc Bras Med Trop* (2001) 34(5):413–9. doi:10.1590/S0037-86822001000500003
7. Sanchez LV, Ramirez JD. Congenital and oral transmission of American trypanosomiasis: an overview of physiopathogenic aspects. *Parasitology* (2013) 140(2):147–59. doi:10.1017/S0031182012001394
8. Noya BA, Diaz-Bello Z, Colmenares C, Ruiz-Guevara R, Mauriello L, Munoz-Calderon A, et al. Update on oral Chagas disease outbreaks in Venezuela: epidemiological, clinical and diagnostic approaches. *Mem Inst Oswaldo Cruz* (2015) 110(3):377–86. doi:10.1590/0074-02760140285
9. Toso MA, Vial UF, Galanti N. [Oral transmission of Chagas' disease]. *Rev Med Chil* (2011) 139(2):258–66.
10. Hotez PJ, Molyneux DH, Stillwaggon E, Bentwich Z, Kumaresan J. Neglected tropical diseases and HIV/AIDS. *Lancet* (2006) 368(9550):1865–6. doi:10.1016/S0140-6736(06)69764-X
11. Savino W, Villa-Verde DM, Mendes-da-Cruz DA, Silva-Monteiro E, Perez AR, Aoki Mdel P, et al. Cytokines and cell adhesion receptors in the regulation of immunity to *Trypanosoma cruzi*. *Cytokine Growth Factor Rev* (2007) 18(1–2):107–24. doi:10.1016/j.cytogfr.2007.01.010
12. Parada H, Carrasco HA, Anez N, Fuenmayor C, Inglessis I. Cardiac involvement is a constant finding in acute Chagas' disease: a clinical, parasitological and histopathological study. *Int J Cardiol* (1997) 60(1):49–54. doi:10.1016/S0167-5273(97)02952-5
13. Devera R, Fernandes O, Coura JR. Should *Trypanosoma cruzi* be called "cruzi" complex? A review of the parasite diversity and the potential of selecting population after in vitro culturing and mice infection. *Mem Inst Oswaldo Cruz* (2003) 98(1):1–12. doi:10.1590/S0074-02762003000100001
14. Punukollu G, Gowda RM, Khan IA, Navarro VS, Vasavada BC. Clinical aspects of the Chagas' heart disease. *Int J Cardiol* (2007) 115(3):279–83. doi:10.1016/j.ijcard.2006.03.004
15. de Meis J, Morrot A, Farias-de-Oliveira DA, Villa-Verde DM, Savino W. Differential regional immune response in Chagas disease. *PLoS Negl Trop Dis* (2009) 3(7):e417. doi:10.1371/journal.pntd.0000417
16. Gonzalez FB, Calmon-Hamaty F, No Seara Cordeiro S, Fernandez Bussy R, Spinelli SV, D'Attilio L, et al. *Trypanosoma cruzi* experimental infection impacts on the thymic regulatory T cell compartment. *PLoS Negl Trop Dis* (2016) 10(1):e0004285. doi:10.1371/journal.pntd.0004285
17. Morrot A, Terra-Granado E, Perez AR, Silva-Barbosa SD, Milicevic NM, Farias-de-Oliveira DA, et al. Chagasic thymic atrophy does not affect negative selection but results in the export of activated CD4+CD8+ T cells in severe forms of human disease. *PLoS Negl Trop Dis* (2011) 5(8):e1268. doi:10.1371/journal.pntd.0001268
18. Giraldo NA, Bolanos NI, Cuellar A, Guzman F, Uribe AM, Bedoya A, et al. Increased CD4+/CD8+ double-positive T cells in chronic Chagasic patients. *PLoS Negl Trop Dis* (2011) 5(8):e1294. doi:10.1371/journal.pntd.0001294
19. Thiault N, Darrigues J, Adoue V, Gros M, Binet B, Perals C, et al. Peripheral regulatory T lymphocytes recirculating to the thymus suppress the development of their precursors. *Nat Immunol* (2015) 16(6):628–34. doi:10.1038/ni.3150
20. Hodge DL, Reynolds D, Cerban FM, Correa SG, Baez NS, Young HA, et al. MCP-1/CCR2 interactions direct migration of peripheral B and T lymphocytes to the thymus during acute infectious/inflammatory processes. *Eur J Immunol* (2012) 42(10):2644–54. doi:10.1002/eji.201242408
21. Sprent J, Surh CD. Re-entry of mature T cells to the thymus: an epiphenomenon? *Immunol Cell Biol* (2009) 87(1):46–9. doi:10.1038/icb.2008.88
22. de Meis J, Aurelio Farias-de-Oliveira D, Nunes Panzenhagen PH, Maran N, Villa-Verde DM, Morrot A, et al. Thymus atrophy and double-positive escape are common features in infectious diseases. *J Parasitol Res* (2012) 2012:574020. doi:10.1155/2012/574020
23. de Meis J, Mendes-da-Cruz DA, Farias-de-Oliveira DA, Correa-de-Santana E, Pinto-Mariz F, Cotta-de-Almeida V, et al. Atrophy of mesenteric lymph nodes in experimental Chagas' disease: differential role of Fas/Fas-L and TNFRI/TNF pathways. *Microbes Infect* (2006) 8(1):221–31. doi:10.1016/j.micinf.2005.06.027
24. Farias-de-Oliveira DA, Cotta-de-Almeida V, Villa-Verde DM, Riederer I, Meis J, Savino W. Fibronectin modulates thymocyte-thymic epithelial cell interactions following *Trypanosoma cruzi* infection. *Mem Inst Oswaldo Cruz* (2013) 108(7):825–31. doi:10.1590/0074-0276130071
25. Leite de Moraes MC, Hontebeyrie-Joskowicz M, Leboulenger F, Savino W, Dardenne M, Lepault F. Studies on the thymus in Chagas' disease. II. Thymocyte subset fluctuations in *Trypanosoma cruzi*-infected mice: relationship to stress. *Scand J Immunol* (1991) 33(3):267–75. doi:10.1111/j.1365-3083.1991.tb01772.x
26. Mendes-da-Cruz DA, de Meis J, Cotta-de-Almeida V, Savino W. Experimental *Trypanosoma cruzi* infection alters the shaping of the central and peripheral T-cell repertoire. *Microbes Infect* (2003) 5(10):825–32. doi:10.1016/S1286-4579(03)00156-4
27. Morrot A, Barreto de Albuquerque J, Berbert LR, de Carvalho Pinto CE, de Meis J, Savino W. Dynamics of lymphocyte populations during *Trypanosoma cruzi* infection: from thymocyte depletion to differential cell expansion/contraction in peripheral lymphoid organs. *J Trop Med* (2012) 2012:747185. doi:10.1155/2012/747185
28. Perez AR, Berbert LR, Lepletier A, Revelli S, Bottasso O, Silva-Barbosa SD, et al. TNF-alpha is involved in the abnormal thymocyte migration during experimental *Trypanosoma cruzi* infection and favors the export of immature cells. *PLoS One* (2012) 7(3):e34360. doi:10.1371/journal.pone.0034360
29. Savino W. The thymus is a common target organ in infectious diseases. *PLoS Pathog* (2006) 2(6):e62. doi:10.1371/journal.ppat.0020062
30. Savino W, Mendes-Da-Cruz DA, Smaniotto S, Silva-Monteiro E, Villa-Verde DM. Molecular mechanisms governing thymocyte migration: combined role of chemokines and extracellular matrix. *J Leukoc Biol* (2004) 75(6):951–61. doi:10.1189/jlb.1003455
31. de Meis J, Ferreira LM, Guillermo LV, Silva EM, Dosreis GA, Lopes MF. Apoptosis differentially regulates mesenteric and subcutaneous lymph node immune responses to *Trypanosoma cruzi*. *Eur J Immunol* (2008) 38(1):139–46. doi:10.1002/eji.200737582
32. Farias-de-Oliveira DA, Villa-Verde DM, Nunes Panzenhagen PH, Silva dos Santos D, Berbert LR, Savino W, et al. Caspase-8 and caspase-9 mediate thymocyte apoptosis in *Trypanosoma cruzi* acutely infected mice. *J Leukoc Biol* (2013) 93(2):227–34. doi:10.1189/jlb.1211589
33. Antunez MI, Feinstein RE, Cardoni RL, Gronvik KO. *Trypanosoma cruzi*: T cell subpopulations in the Peyer's patches of BALB/c infected mice. *Exp Parasitol* (1997) 87(1):58–64. doi:10.1006/expr.1997.4171
34. Minoprio PM, Coutinho A, Joskowicz M, D'Imperio Lima MR, Eisen H. Polyclonal lymphocyte responses to murine *Trypanosoma cruzi* infection. II. Cytotoxic T lymphocytes. *Scand J Immunol* (1986) 24(6):669–79. doi:10.1111/j.1365-3083.1986.tb02186.x
35. Minoprio PM, Eisen H, Forni L, D'Imperio Lima MR, Joskowicz M, Coutinho A. Polyclonal lymphocyte responses to murine *Trypanosoma cruzi* infection. I. Quantitation of both T- and B-cell responses. *Scand J Immunol* (1986) 24(6):661–8. doi:10.1111/j.1365-3083.1986.tb02185.x
36. Todeschini AR, Nunes MP, Pires RS, Lopes MF, Previato JO, Mendonca-Previato L, et al. Costimulation of host T lymphocytes by a trypanosomal trans-sialidase: involvement of CD43 signaling. *J Immunol* (2002) 168(10):5192–8. doi:10.4049/jimmunol.168.10.5192
37. Gao W, Wortis HH, Pereira MA. The *Trypanosoma cruzi* trans-sialidase is a T cell-independent B cell mitogen and an inducer of non-specific Ig secretion. *Int Immunol* (2002) 14(3):299–308. doi:10.1093/intimm/14.3.299

38. Reina-San-Martin B, Degrave W, Rougeot C, Cosson A, Chamond N, Cordeiro-Da-Silva A, et al. A B-cell mitogen from a pathogenic trypanosome is a eukaryotic proline racemase. *Nat Med* (2000) 6(8):890–7. doi:10.1038/78651
39. Bryan MA, Norris KA. Genetic immunization converts the *Trypanosoma cruzi* B-cell mitogen proline racemase to an effective immunogen. *Infect Immun* (2010) 78(2):810–22. doi:10.1128/IAI.00926-09
40. Buschiazzo A, Goytia M, Schaeffer F, Degrave W, Shepard W, Gregoire C, et al. Crystal structure, catalytic mechanism, and mitogenic properties of *Trypanosoma cruzi* proline racemase. *Proc Natl Acad Sci U S A* (2006) 103(6):1705–10. doi:10.1073/pnas.0509010103
41. Hurtado-Alvarado G, Pavon L, Castillo-Garcia SA, Hernandez ME, Dominguez-Salazar E, Velazquez-Moctezuma J, et al. Sleep loss as a factor to induce cellular and molecular inflammatory variations. *Clin Dev Immunol* (2013) 2013:801341. doi:10.1155/2013/801341
42. Savino W, Mendes-da-Cruz DA, Lepletier A, Dardenne M. Hormonal control of T-cell development in health and disease. *Nat Rev Endocrinol* (2016) 12(2):77–89. doi:10.1038/nrendo.2015.168
43. e Silva PM, Carvalho VF, Cordeiro RS, Martins MA. Down-regulation of allergic responses in conditions of experimental diabetes: a role for glucocorticoids? *Neuroimmunomodulation* (2009) 16(1):13–8. doi:10.1159/000179662
44. Haddad JJ, Saade NE, Safieh-Garabedian B. Cytokines and neuro-immune-endocrine interactions: a role for the hypothalamic-pituitary-adrenal revolving axis. *J Neuroimmunol* (2002) 133(1–2):1–19. doi:10.1016/S0165-5728(02)00357-0
45. Nova CC. entidade mórbida do homem. *Mem Inst Oswaldo Cruz* (1911) 3:219–75.
46. Bestetti RB, Cardinalli-Neto A, Restini CB, Couto LB. Could Carlos Chagas' assumption on the relationship between goiter and chronic Chagas heart disease be correct? A historical reappraisal. *Int J Cardiol* (2016) 202:410–2. doi:10.1016/j.ijcard.2015.09.052
47. Kanczkowski W, Sue M, Zacharowski K, Reincke M, Bornstein SR. The role of adrenal gland microenvironment in the HPA axis function and dysfunction during sepsis. *Mol Cell Endocrinol* (2015) 408:241–8. doi:10.1016/j.mce.2014.12.019
48. Roggero E, Perez AR, Tamae-Kakazu M, Piazzon I, Nepomnaschy I, Besedovsky HO, et al. Endogenous glucocorticoids cause thymus atrophy but are protective during acute *Trypanosoma cruzi* infection. *J Endocrinol* (2006) 190(2):495–503. doi:10.1677/joe.1.06642
49. Villar SR, Ronco MT, Fernandez Bussy R, Roggero E, Lepletier A, Manarin R, et al. Tumor necrosis factor-alpha regulates glucocorticoid synthesis in the adrenal glands of *Trypanosoma cruzi* acutely-infected mice. the role of TNF-R1. *PLoS One* (2013) 8(5):e63814. doi:10.1371/journal.pone.0063814
50. Correa-de-Santana E, Paez-Pereda M, Theodoropoulou M, Kenji Nihei O, Gruebler Y, Bozza M, et al. Hypothalamus-pituitary-adrenal axis during *Trypanosoma cruzi* acute infection in mice. *J Neuroimmunol* (2006) 173(1–2):12–22. doi:10.1016/j.jneuroim.2005.08.015
51. Bornstein SR, Ziegler CG, Krug AW, Kanczkowski W, Rettori V, McCann SM, et al. The role of toll-like receptors in the immune-adrenal crosstalk. *Ann N Y Acad Sci* (2006) 1088:307–18. doi:10.1196/annals.1366.027
52. Bafica A, Santiago HC, Goldszmid R, Ropert C, Gazzinelli RT, Sher A. Cutting edge: TLR9 and TLR2 signaling together account for MyD88-dependent control of parasitemia in *Trypanosoma cruzi* infection. *J Immunol* (2006) 177(6):3515–9. doi:10.4049/jimmunol.177.6.3515
53. Tran N, Koch A, Berkels R, Boehm O, Zacharowski PA, Baumgarten G, et al. Toll-like receptor 9 expression in murine and human adrenal glands and possible implications during inflammation. *J Clin Endocrinol Metab* (2007) 92(7):2773–83. doi:10.1210/jc.2006-2697
54. Rodrigues MM, Oliveira AC, Bellio M. The immune response to *Trypanosoma cruzi*: role of toll-like receptors and perspectives for vaccine development. *J Parasitol Res* (2012) 2012:507874. doi:10.1155/2012/507874
55. Perez AR, Roggero E, Nicora A, Palazzi J, Besedovsky HO, Del Rey A, et al. Thymus atrophy during *Trypanosoma cruzi* infection is caused by an immuno-endocrine imbalance. *Brain Behav Immun* (2007) 21(7):890–900. doi:10.1016/j.bbi.2007.02.004
56. Bornstein SR, Engeland WC, Ehrhart-Bornstein M, Herman JP. Dissociation of ACTH and glucocorticoids. *Trends Endocrinol Metab* (2008) 19(5):175–80. doi:10.1016/j.tem.2008.01.009
57. Savino W. Prolactin: an immunomodulator in health and disease. *Front Horm Res* (2017) 48:69–75. doi:10.1159/000452906
58. Weigent DA. Lymphocyte GH-axis hormones in immunity. *Cell Immunol* (2013) 285(1–2):118–32. doi:10.1016/j.cellimm.2013.10.003
59. Torner L. Actions of prolactin in the brain: from physiological adaptations to stress and neurogenesis to psychopathology. *Front Endocrinol* (2016) 7:25. doi:10.3389/fendo.2016.00025
60. Peroski M, Proudan N, Grignol G, Merchenthaler I, Dudas B. Corticotropin-releasing hormone (CRH)-immunoreactive (IR) axon varicosities target a subset of growth hormone-releasing hormone (GHRH)-IR neurons in the human hypothalamus. *J Chem Neuroanat* (2016) 78:119–24. doi:10.1016/j.jchemneu.2016.09.005
61. de Mello-Coelho V, Gagnerault MC, Souberbielle JC, Strasburger CJ, Savino W, Dardenne M, et al. Growth hormone and its receptor are expressed in human thymic cells. *Endocrinology* (1998) 139(9):3837–42. doi:10.1210/endo.139.9.6199
62. Correa-de-Santana E, Paez-Pereda M, Theodoropoulou M, Renner U, Stalla J, Stalla GK, et al. Modulation of growth hormone and prolactin secretion in *Trypanosoma cruzi*-infected mammosomatotrophic cells. *Neuroimmunomodulation* (2009) 16(3):208–12. doi:10.1159/000205513
63. Long RG, Albuquerque RH, Prata A, Barnes AJ, Adrian TE, Christofides ND, et al. Response of plasma pancreatic and gastrointestinal hormones and growth hormone to oral and intravenous glucose and insulin hypoglycaemia in Chagas's disease. *Gut* (1980) 21(9):772–7. doi:10.1136/gut.21.9.772
64. Lepletier A, de Frias Carvalho V, Morrot A, Savino W. Thymic atrophy in acute experimental Chagas disease is associated with an imbalance of stress hormones. *Ann N Y Acad Sci* (2012) 1262:45–50. doi:10.1111/j.1749-6632.2012.06601.x
65. Lepletier A, de Carvalho VF, Rodrigues e Silva PM, Villar S, Perez AR, Savino W, et al. *Trypanosoma cruzi* disrupts thymic homeostasis by altering intrathymic and systemic stress-related endocrine circuitries. *PLoS Negl Trop Dis* (2013) 7(11):e2470. doi:10.1371/journal.pntd.0002470
66. Perez AR, Silva-Barbosa SD, Berbert LR, Revelli S, Beloscar J, Savino W, et al. Immunoneuroendocrine alterations in patients with progressive forms of chronic Chagas disease. *J Neuroimmunol* (2011) 235(1–2):84–90. doi:10.1016/j.jneuroim.2011.03.010
67. Perez AR, Bertoya AA, Revelli S, Garcia F. A high corticosterone/DHEA-s ratio in young rats infected with *Trypanosoma cruzi* is associated with increased susceptibility. *Mem Inst Oswaldo Cruz* (2011) 106(4):416–23. doi:10.1590/S0074-02762011000400006
68. Moreira A, Napimoga MH, Benatti BB, Silva GA, Rocha-Rodrigues DB, Clemente-Napimoga JT, et al. Morphological changes and EGF expression in the granular convoluted tubule cells of submandibular glands of *Trypanosoma cruzi* infected rats. *Tissue Cell* (2008) 40(4):293–8. doi:10.1016/j.tice.2008.02.004
69. de Araujo FF, Vitelli-Avelar DM, Teixeira-Carvalho A, Antas PR, Assis Silva Gomes J, Sathler-Avelar R, et al. Regulatory T cells phenotype in different clinical forms of Chagas' disease. *PLoS Negl Trop Dis* (2011) 5(5):e992. doi:10.1371/journal.pntd.0000992
70. Fiuza JA, Fujiwara RT, Gomes JA, Rocha MO, Chaves AT, de Araujo FF, et al. Profile of central and effector memory T cells in the progression of chronic human chagas disease. *PLoS Negl Trop Dis* (2009) 3(9):e512. doi:10.1371/journal.pntd.0000512
71. Gironès N, Cuervo H, Fresno M. Trypanosoma cruzi-induced molecular mimicry and Chagas' disease. In: Oldstone MB, editor. *Molecular Mimicry: Infection-Inducing Autoimmune Disease. Current Topics in Microbiology and Immunology*. (Vol 296), Berlin, Heidelberg: Springer (2005).
72. Oliveira EC, Fujisawa MM, Hallal Longo DE, Farias AS, Contin Moraes J, Guariento ME, et al. Neuropathy of gastrointestinal Chagas' disease: immune response to myelin antigens. *Neuroimmunomodulation* (2009) 16(1):54–62. doi:10.1159/000179667
73. Albareda MC, Laucella SA. Modulation of *Trypanosoma cruzi*-specific T-cell responses after chemotherapy for chronic Chagas disease. *Mem Inst Oswaldo Cruz* (2015) 110(3):414–21. doi:10.1590/0074-02760140386
74. Lepletier A, de Almeida L, Santos L, da Silva Sampaio L, Paredes B, Gonzalez FB, et al. Early double-negative thymocyte export in *Trypanosoma cruzi* infection is restricted by sphingosine receptors and associated with human chagas disease. *PLoS Negl Trop Dis* (2014) 8(10):e3203. doi:10.1371/journal.pntd.0003203

75. Carbajosa S, Gea S, Chillon-Marinas C, Poveda C, Del Carmen Maza M, Fresno M, et al. Altered bone marrow lymphopoiesis and interleukin-6-dependent inhibition of thymocyte differentiation contribute to thymic atrophy during *Trypanosoma cruzi* infection. *Oncotarget* (2017) 8(11):17551–61. doi:10.18632/oncotarget.14886
76. Savino W. Neuroendocrine control of T cell development in mammals: role of growth hormone in modulating thymocyte migration. *Exp Physiol* (2007) 92(5):813–7. doi:10.1113/expphysiol.2007.038422
77. Mendes-da-Cruz DA, Silva JS, Cotta-de-Almeida V, Savino W. Altered thymocyte migration during experimental acute *Trypanosoma cruzi* infection: combined role of fibronectin and the chemokines CXCL12 and CCL4. *Eur J Immunol* (2006) 36(6):1486–93. doi:10.1002/eji.200535629
78. Perez AR, Bottasso O, Savino W. The impact of infectious diseases upon neuroendocrine circuits. *Neuroimmunomodulation* (2009) 16(2):96–105. doi:10.1159/000180264
79. Perez AR, Silva-Barbosa SD, Roggero E, Calmon-Hamaty F, Villar SR, Gutierrez FR, et al. Immunoendocrinology of the thymus in Chagas disease. *Neuroimmunomodulation* (2011) 18(5):328–38. doi:10.1159/000329494
80. Savino W, Dardenne M. Neuroendocrine control of thymus physiology. *Endocr Rev* (2000) 21(4):412–43. doi:10.1210/edrv.21.4.0402
81. Roggero E, Perez A, Tamae-Kakazu M, Piazzon I, Nepomnaschy I, Wietzerbin J, et al. Differential susceptibility to acute *Trypanosoma cruzi* infection in BALB/c and C57BL/6 mice is not associated with a distinct parasite load but cytokine abnormalities. *Clin Exp Immunol* (2002) 128(3):421–8. doi:10.1046/j.1365-2249.2002.01874.x
82. Vacchio MS, Papadopoulos V, Ashwell JD. Steroid production in the thymus: implications for thymocyte selection. *J Exp Med* (1994) 179(6):1835–46. doi:10.1084/jem.179.6.1835
83. Qiao S, Chen L, Okret S, Jondal M. Age-related synthesis of glucocorticoids in thymocytes. *Exp Cell Res* (2008) 314(16):3027–35. doi:10.1016/j.yexcr.2008.06.014
84. Da Costa SC, Calabrese KS, Bauer PG, Savino W, Lagrange PH. Studies of the thymus in Chagas' disease: III. Colonization of the thymus and other lymphoid organs of adult and newborn mice by *Trypanosoma cruzi*. *Pathol Biol (Paris).* (1991) 39(2):91–7.
85. De Mello-Coelho V, Savino W, Postel-Vinay MC, Dardenne M. Role of prolactin and growth hormone on thymus physiology. *Dev Immunol* (1998) 6(3–4):317–23. doi:10.1155/1998/89782
86. Hattori N. Expression, regulation and biological actions of growth hormone (GH) and ghrelin in the immune system. *Growth Horm IGF Res* (2009) 19(3):187–97. doi:10.1016/j.ghir.2008.12.001
87. Dorshkind K, Horseman ND. The roles of prolactin, growth hormone, insulin-like growth factor-I, and thyroid hormones in lymphocyte development and function: insights from genetic models of hormone and hormone receptor deficiency. *Endocr Rev* (2000) 21(3):292–312. doi:10.1210/edrv.21.3.0397
88. Smaniotto S, Ribeiro-Carvalho MM, Dardenne M, Savino W, de Mello-Coelho V. Growth hormone stimulates the selective trafficking of thymic CD4+CD8- emigrants to peripheral lymphoid organs. *Neuroimmunomodulation* (2004) 11(5):299–306. doi:10.1159/000079410
89. Smaniotto S, de Mello-Coelho V, Villa-Verde DM, Pleau JM, Postel-Vinay MC, Dardenne M, et al. Growth hormone modulates thymocyte development in vivo through a combined action of laminin and CXC chemokine ligand 12. *Endocrinology* (2005) 146(7):3005–17. doi:10.1210/en.2004-0709
90. Savino W, Dardenne M. Pleiotropic modulation of thymic functions by growth hormone: from physiology to therapy. *Curr Opin Pharmacol* (2010) 10(4):434–42. doi:10.1016/j.coph.2010.04.002
91. Loria RM. Antiglucocorticoid function of androstenetriol. *Psychoneuroendocrinology* (1997) 22(Suppl 1):S103–8. doi:10.1016/S0306-4530(97)00005-X
92. do Prado junior JC, Leal Mde P, Anselmo-Franci JA, de Andrade juniur HF, Kloetzel JK. Influence of female gonadal hormones on the parasitemia of female *Calomys callosus* infected with the "Y" strain of *Trypanosoma cruzi*. *Parasitol Res* (1998) 84(2):100–5. doi:10.1007/s004360050364
93. do Prado JC Jr, Levy AM, Leal MP, Bernard E, Kloetzel JK. Influence of male gonadal hormones on the parasitemia and humoral response of male *Calomys callosus* infected with the Y strain of *Trypanosoma cruzi*. *Parasitol Res* (1999) 85(10):826–9. doi:10.1007/s004360050639
94. Filipin Mdel V, Brazao V, Caetano LC, Santello FH, Toldo MP, Caetano LN, et al. *Trypanosoma cruzi*: orchiectomy and dehydroepiandrosterone therapy in infected rats. *Exp Parasitol* (2008) 120(3):249–54. doi:10.1016/j.exppara.2008.07.012
95. Mucci J, Mocetti E, Leguizamon MS, Campetella O. A sexual dimorphism in intrathymic sialylation survey is revealed by the trans-sialidase from *Trypanosoma cruzi*. *J Immunol* (2005) 174(8):4545–50. doi:10.4049/jimmunol.174.8.4545
96. Filipin Mdel V, Caetano LC, Brazao V, Santello FH, Toldo MP, do Prado JC Jr. DHEA and testosterone therapies in *Trypanosoma cruzi*-infected rats are associated with thymic changes. *Res Vet Sci* (2010) 89(1):98–103. doi:10.1016/j.rvsc.2010.01.016
97. Manarin R, Villar SR, Fernandez Bussy R, Gonzalez FB, Deschutter EV, Bonantini AP, et al. Reciprocal influences between leptin and glucocorticoids during acute *Trypanosoma cruzi* infection. *Med Microbiol Immunol* (2013) 202(5):339–52. doi:10.1007/s00430-013-0294-1
98. Procaccini C, De Rosa V, Galgani M, Carbone F, Cassano S, Greco D, et al. Leptin-induced mTOR activation defines a specific molecular and transcriptional signature controlling CD4+ effector T cell responses. *J Immunol* (2012) 189(6):2941–53. doi:10.4049/jimmunol.1200935
99. Sreenivasan J, Schlenner S, Franckaert D, Dooley J, Liston A. The thymoprotective function of leptin is indirectly mediated via suppression of obesity. *Immunology* (2015) 146(1):122–9. doi:10.1111/imm.12488
100. Gruver AL, Ventevogel MS, Sempowski GD. Leptin receptor is expressed in thymus medulla and leptin protects against thymic remodeling during endotoxemia-induced thymus involution. *J Endocrinol* (2009) 203(1):75–85. doi:10.1677/JOE-09-0179
101. Nagajyothi F, Zhao D, Machado FS, Weiss LM, Schwartz GJ, Desruisseaux MS, et al. Crucial role of the central leptin receptor in murine *Trypanosoma cruzi* (Brazil strain) infection. *J Infect Dis* (2010) 202(7):1104–13. doi:10.1086/656189
102. Cabalen ME, Cabral MF, Sanmarco LM, Andrada MC, Onofrio LI, Ponce NE, et al. Chronic *Trypanosoma cruzi* infection potentiates adipose tissue macrophage polarization toward an anti-inflammatory M2 phenotype and contributes to diabetes progression in a diet-induced obesity model. *Oncotarget* (2016) 7(12):13400–15. doi:10.18632/oncotarget.7630
103. Barbosa-Ferreira JM, Mady C, Ianni BM, Lopes HF, Ramires FJ, Salemi VM, et al. Dysregulation of autonomic nervous system in Chagas' heart disease is associated with altered adipocytokines levels. *PLoS One* (2015) 10(7):e0131447. doi:10.1371/journal.pone.0131447
104. Manarin R, Lamas MC, Bottasso E, Serra E, Revelli S, Salomon CJ. Efficacy of novel benznidazole solutions during the experimental infection with *Trypanosoma cruzi*. *Parasitol Int* (2013) 62(1):79–81. doi:10.1016/j.parint.2012.09.001
105. Pérez AR, Morrot A, Berbert LR, Terra-Granado E, Savino W. Extrathymic CD4+CD8+ lymphocytes in Chagas disease: possible relationship with an immunoendocrine imbalance. *Ann N Y Acad Sci* (2012) 1262:27–36. doi:10.1111/j.1749-6632.2012.06627.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor is currently co-organizing a Research Topic with the authors AP, WS and confirms the absence of any other collaboration.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership