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Local glucocorticoid production in the thymus

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ABSTRACT

Besides generating immunocompetent T lymphocytes, the thymus is an established site of *de novo* extra-adrenal glucocorticoid (GC) production. Among the compartments of the thymus, both stromal thymic epithelial cells (TECs) and thymocytes secrete biologically active GCs. Locally produced GCs secreted by the various thymic cellular compartments have been suggested to have different impact on thymic homeostasis. TEC-derived GCs may regulate thymocyte differentiation whereas thymocyte-derived GCs might regulate age-dependent involution. However the full biological significance of thymic-derived GCs is still not fully understood. In this review, we summarize and describe recent advances in the understanding of local GC production in the thymus and immunoregulatory steroid production by peripheral T cells and highlight the possible role of local GCs for thymus function.

1. Introduction

The notion and knowledge of possible significance of extra-adrenally synthesized glucocorticoids (GCs) are growing. The thymus was the first extra-adrenal organ where expression of steroidogenic enzymes and subsequently *de novo* GC synthesis was demonstrated [1]. Besides the thymus, there is clear evidence that additional organs have the capacity for *de novo* synthesis of GCs, including the skin, intestine, brain (extra-adrenal GC-production reviewed and summarized in [2,3]) and recently reported fat deposits [4]. With regard to the thymus, the functions of the thymus-derived *de novo* synthesized GCs have been suggested to involve induction of apoptosis of developing thymocytes or alternatively to induce their survival by modifying the outcome of the T cell antigen receptor-induced signals [5,6].

GCs execute their effects via a nuclear receptor, the glucocorticoid receptor (GR). Upon ligand binding, the GR dissociates from a chaperone complex in the cytoplasm where after it homodimerizes, translocates to the nucleus and modulates gene expression by binding to target genes via so called glucocorticoid responsive elements (GRE) or by interacting with other transcription factors modifying their effects [7]. Alternatively, the GR may act via non-genomic mechanisms as exemplified by GR binding to adaptor proteins of the T cell antigen receptor signaling machinery [8]. Importantly and in relation to the formation of biologically active GCs, inactive GC precursors can be secreted and circulated and enzymatically converted to biologically active steroids by 11β-hydroxy-steroid dehydrogenase type I (11β-HSD1) enzyme in peripheral organs [9]. 11β-HSD1 is expressed in the thymus and has been shown to be able to amplify GC actions in the thymus [10]. However, the exact role of the conversion of inactive to active GCs in the regulation of thymus homeostasis is still unknown.

GCs have well known immunoregulatory effects on the immune system, including the thymus. High doses of GCs e.g. following stress or administration, are known to induce thymus involution due to thymocyte and TEC depletion [11,12]. GCs, whether systemic or local, have been suggested to affect the generation of the T cell repertoire. In line with this, a recent report showed that signaling through the glucocorticoid receptor (GR) was needed to maintain proper T cell response and thus, immunological fitness. This study which used conditional GR knockout mice in the T cell

Abbreviations: ACTH, adrenocorticotropic hormone; DN, double negative; Dll, delta-like ligand; DP, double positive; HPA-axis, hypothalamus-pituitary-adrenal axis; HSD, hydroxysteroid-dehydrogenase; IL, interleukin; SP, single positive; IFN, interferon; TEC, thymic epithelial cell; GC, glucocorticoid; GR, glucocorticoid receptor; GRE, glucocorticoid responsive elements; MAPK, mitogen activated protein kinase; MHC, major histocompatibility complex; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor κB; PKB, protein kinase B; PKC, protein kinase C; TCR, T cell receptor.

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lineage, revealed that T cells from these mice possessed a less competent repertoire and were less able to challenge antigens derived from virus [13]. Other studies using knockout mice with ablated GR expression in thymocytes did not observe an impact on thymocyte development, however, the influence on T cell repertoire and antigen response was not studied in detail [14–17]. Though, in most of cases, mice with deletion of GR exon 2 (and partially exon 1) mice were generated and used, which does not result in complete ablation of GR expression but rather of expression of N-terminal deleted GR variants with partial GR activity, thus only leading to mild phenotype alterations. Recently, mice with GR exon 2 and 3, respectively, conditional knockouts in hypothalamic neurons were characterized and compared. Mice with GR exon 3 deletion, encompassing the first Zn-finger in the DNA binding domain, resulted in a more robust phenotype suggesting that data derived from GR exon 3 knockout mice should be regarded as more relevant when evaluating the phenotype following ablated GR expression [18]. Furthermore, throughout the years, conflicting results regarding the impact of GCs in transgenic mice with increased or decreased GC sensitivity due to overexpressed or downregulated GR expression in thymocytes on thymocyte homeostasis have been published, suggesting that the role of GCs on thymus is complex [19,20]. In addition, in these reports the relative role of locally synthesized *vs.* systemic GCs in these processes was not determined. Furthermore, the complexity of thymus homeostasis following GC or GR manipulations, particularly *in vivo*, might be indirect effect via changes in the hypothalamic–pituitary-adrenal (HPA)-axis. As an example, we recently showed that adrenocorticotropic hormone (ACTH) has a direct tropic effect on the thymus, which to a large part explains the enlargement of the thymus when systemic GC concentrations are low as e.g. after adrenalectomy [21].

Here, we review current knowledge of thymus-derived GCs and their possible role for thymus homeostasis and T cell differentiation. In addition, we highlight the growing evidence of extrathymic T cell-derived immunoregulatory steroids and their possible implications for immune homeostasis.

2. Overview of the structure and function of the thymus and fundamentals of thymic T cell selection

The thymus is the primary site where T lymphocytes, cells part of the adaptive immune system, undergo differentiation and complete their maturation. The thymus main micro anatomical regions are the cortex and the medulla, both of which have distinct resident cellular constituents and well defined functions. The thymus has an intricate developmental pattern and grows until puberty, after which it starts to involute. This age-associated involution corresponds to thymic epithelial cell degeneration and the replacement of the functional lymphoid thymic tissue by fat cells [22,23]. In addition to the age-dependent involution of the thymus, thymic involution can be induced by several conditions and factors: among them pregnancy, drugs (chemotherapy, GCs, cytostatic drugs), irradiation, cancer, severe infections or graft versus host disease [24]. Both the age-dependent and more acute involution lead to a significant decrease in naïve T cell output and a decrease in the number of peripheral naïve T cells with a resulting decline in effective immune responses. The decline in seeding of new naïve T cell into the periphery has been associated with the increased incidence of infection, autoimmunity and possibly cancer in the elderly population [25]. The main constituents of the thymus are stromal elements and lymphoid cells. Among the stromal elements, thymic epithelial cells (TECs) [26] and dendritic cells are the most important [27]. In the last decade, new functions and developmental relationships of cortical and medullar TECs have been identified. TECs, as part of a complex three-dimensional compartmentalized network within the thymus, are crucial players in maintaining T cell development by providing soluble factors and various cell surface molecules to promote proliferation, migration and selection of thymocytes [28]. The TECs are key cells in antigenic peptide presentation via the major histocompatibility complexes (MHC) for developing thymocytes. Recently it has been shown that thymocyte positive selection is mediated in the thymic cortex by the cortical thymic epithelium [29]. Besides presenting antigens to developing thymocytes, TECs also secrete cytokines (among them IL-7) [30] and Wnt-molecules [31]) and express cell surface molecules indispensable for thymocyte development (e.g. Dll4 on TECs that interacts with Notch1 expressed on thymocytes) [32]. In the medulla, medullary TECs (mTECs) ensure that T cells recognize the body's own antigens by facilitating the expression of tissue-related antigens and present them to developing thymocytes [33,34]. The thymic medulla is also the site where central T cell tolerance develops, thus, shaping the T cell repertoire and removing the harmful and autoreactive T cell clones in order to prevent autoimmunity and subsequent organ destruction [35]. Taken together, the thymus is responsible for functional, immunologically competent T cell output and to ensure that the T cells exported to the periphery are tolerant to self-antigens and responsive enough against non-self-antigens.

Thymocyte development from immature cells to naïve T cells ready to be seeded to the periphery occurs through well-defined stages. Each stage can be characterized through acquisition and loss of cell surface molecules and stage-specific transcription factors. First, T cell precursors from the bone marrow, defined as CD3- and CD4-CD8- double negative (DN) T cells, colonize the cortico-medullary junction. These cells undergo rapid proliferation, mainly governed by IL-7 in the thymic cortex, and migrate through the cortex towards the medulla in response to various chemokine signals [36]. Cells that fail to undergo beta-selection and thereby the rearrangement of their antigen receptor genes will undergo apoptosis. Rearrangement of the antigen receptor genes is crucial to gain a T cell repertoire against a broad set of antigens [37]. After acquisition of both CD4 and CD8 co-receptors, together with the gene rearrangement of the TCR genes, the thymocytes defined as CD4+ CD8+ double positive (DP) cells will start to undergo the selection process [38]. First, during positive selection, cell with nonfunctional antigen receptors which do not receive any surviving signals by the TCR-antigen complex will be deleted by apoptosis, a process called death by neglect. This occurs to the vast majority of the DP cells. One of the most remarkable features of DP cells is that they are the most sensitive thymocyte population to apoptotic-inducing stimuli [39]. This includes GC-induced apoptosis, despite the fact that DP cells express a lower level of GR compared to thymocytes in other developmental stages [40,41]. Next, the surviving cells which at this stage have lost either CD4 or CD8 and have become single positive (SP) cells, undergo the negative selection where cells with high affinity antigen receptor for self-antigens are deleted by activation-induced cell death. This process prevents the generation of autoreactive cells that might cause autoimmunity although some autoreactive cells might escape the negative selection causing autoimmune disease. The thymus is also the site for development of regulatory T cells (T_{regs}) [42]. T_{regs} regulate immune responses be secreting various immunoregulatory cytokines to prevent autoimmunity [43]. Finally, when the maturation is successfully completed, CD4+ SP and CD8+ SP naïve T cells [44,45] leave the thymus relocating to various lymphoid as well as non-lymphoid organs where they will fulfill their effector functions. Considering the fact the thymocytes are very sensitive to GCs, locally produced GCs may have an important role in regulating thymus homeostasis and thymocyte differentiation.