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Glucocorticoids in T cell development, differentiation and function

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Abstract | Glucocorticoids (GCs) are small lipid hormones produced by the adrenals that maintain organismal homeostasis. Circadian and stress-induced changes in systemic GC levels regulate metabolism, cardiovascular and neural function, reproduction and immune activity. Our understanding of GC effects on immunity comes largely from administration of exogenous GCs to treat immune or inflammatory disorders. However, it is increasingly clear that endogenous GCs both promote and suppress T cell immunity. Examples include selecting an appropriate repertoire of T cell receptor (TCR) self-affinities in the thymus, regulating T cell trafficking between anatomical compartments, suppressing type 1 T helper (T_H1) cell responses while permitting T_H2 cell and, especially, IL-17-producing T helper cell responses, and promoting memory T cell differentiation and maintenance. Furthermore, in addition to functioning at a distance, extra-adrenal (local) production allows GCs to act as paracrine signals, specifically targeting activated T cells in various contexts in the thymus, mucosa and tumours. These pleiotropic effects on different T cell populations during development and immune responses provide a nuanced understanding of how GCs shape immunity.

Glucocorticoids (GCs) are adrenal-derived, lipid-soluble steroid hormones that circulate in the blood and have pleiotropic effects on the body. They act by binding an intracellular receptor — the glucocorticoid receptor (GR), which is a ubiquitously expressed ligand-dependent transcription factor — and act as systemic regulators of homeostasis. GC signalling in early life is critical for organ development and growth[1]; circadian changes in GC production regulate metabolism and neural function[2,3]; and stress-induced secretion of GCs rapidly mobilizes energy stores, increases cardiovascular output and enhances neural function[4]. GCs have parallel actions on T cells: during early life, GCs programme the T cell receptor (TCR) repertoire; circadian cycles in GC production regulate T cell trafficking and responsiveness; and elevated GC levels during infection prime, direct and control effector and memory T cell responses. In this Review we provide an overview of how GCs function at each of these steps.

GC production and signalling

Glucocorticoid production. Adrenal GC synthesis fluctuates with ultradian (<24 h) and circadian (24 h) rhythms[5,6] and increases dramatically in response to a broad range of stressors. Synthesis is regulated by a neuroendocrine regulatory circuit, the hypothalamus–pituitary–adrenal axis, which drives adrenal expression of multiple enzymes that act in series to convert cholesterol into active GCs: corticosterone in rodents and cortisol in humans[7] (FIG. 1). The adrenals secrete GCs into the blood in which they are carried throughout the body, a textbook model for classical endocrine signalling in which a hormone produced at one site acts on distant targets. As they are lipophilic, GCs are primarily transported in association with blood-borne proteins. The majority of the blood GC content (70–90%) is specifically and saturably bound to corticosteroid-binding globulin (CBG). The remainder is non-specifically and non-saturably associated with proteins such as albumin (5–10%) and erythrocyte membranes (5–10%), or is free in the plasma and, thus, bioavailable (5–10%)[8–10]. In this way, the majority of circulating GC molecules are sequestered and only a small fraction can enter target tissues and cells[11].

Adrenal GCs are regulators of systemic homeostasis, signalling in cells throughout the body. However, GC synthetic enzymes are also expressed in other tissues, especially in response to inflammatory stimuli[12]. All of the enzymes required for GC synthesis are expressed in epithelial cells of the thymus[13,14], intestine[15] and skin[16,17], and extra-adrenal GCs appear to act as paracrine regulators of immune activation. Additionally, cells may express 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), which converts inactive GC metabolites into active GCs, effectively recycling GCs to amplify their activity in a tissue-specific and

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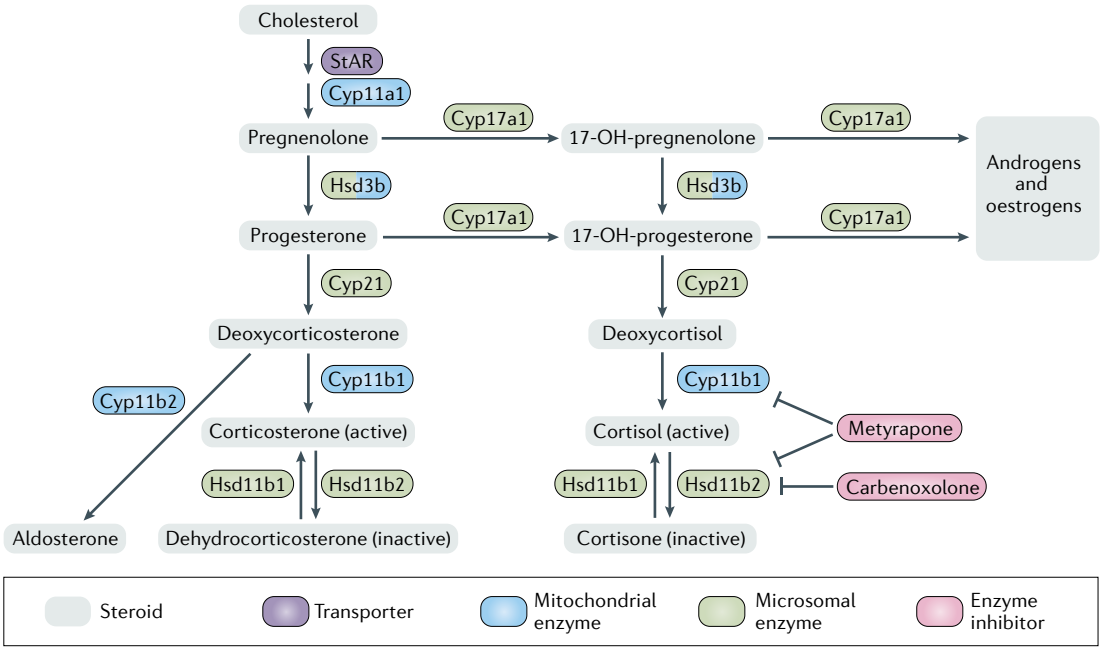

Fig. 1 | **Glucocorticoid synthetic pathway.** Glucocorticoids (GCs) are synthesized from cholesterol via the stepwise activity of a cascade of mitochondrial enzymes (shown in blue) and microsomal enzymes (shown in green). Cholesterol is transported by steroidogenic acute regulatory protein (StAR; shown in purple) to the inner mitochondrial membrane, where it is converted by P450scc (Cyp11a1) into pregnenolone, the precursor to all other steroids. The further activities of 3β-HSD (Hsd3b), P450c21 (Cyp21) and P450c11β (Cyp11b1) then result in synthesis of the active GCs, corticosterone in rodents and cortisol in primates. 11β-Hydroxysteroid dehydrogenase type 2 (11β-HSD2; Hsd11b2) converts active GCs into the inactive metabolites dehydrocorticosterone or cortisone, whereas 11β-HSD1 (Hsd11b1) converts these metabolites into active GCs. Metyrapone blocks the activities of P450c11β, 11β-HSD1 and 11β-HSD2 whereas carbenoxolone (and other glycyrrhetinic acid-related molecules) blocks the activities of 11β-HSD1 and 11β-HSD2. Both inhibitors (shown in pink) are approved for clinical use.

cell-specific manner[18,19]. A combination of endocrine, paracrine and autocrine GC signalling thus results in multilevel regulation of cell-specific GC exposure in a given target cell.

Glucocorticoid receptors. GCs bind GRs (encoded by *Nr3c1*) and mineralocorticoid receptors (encoded by *Nr3c2*). The GR is ubiquitously expressed and binds GCs with moderate affinity (K_d ~ 10 nM), whereas mineralocorticoid receptors have limited distribution and bind GCs with high affinity (K_d ~ 1 nM). In this way, mineralocorticoid receptors are occupied at low GC levels and GRs are occupied when GC levels increase (for example, at the circadian peak or in response to stress). In the kidneys and colon, GCs are metabolized (inactivated) by 11β-HSD2, ensuring that mineralocorticoid receptors specifically respond to the steroid aldosterone (K_d ~ 1 nM) even though circulating aldosterone levels are 1,000-fold lower than GC levels. Thus, the presence or absence of 11β-HSD2 determines whether the mineralocorticoid receptor functions as a receptor for GCs or for aldosterone. Hereafter, we focus on the GR, as T cells express little mineralocorticoid receptor[20].

The GR is a member of the nuclear receptor superfamily, which includes the mineralocorticoid receptor and other steroid (progesterone, androgen and oestrogen) receptors. These ligand-dependent transcription factors have highly conserved structures: an amino-terminal transcriptional activation domain, a DNA-binding domain that recognizes a specific DNA consensus sequence and a ligand-binding domain. The GR-encoding *Nr3c1* gene has nine exons that can generate multiple splice variants. In humans, the classical GRα is a 777 amino acid protein. GRβ is the product of an alternatively spliced transcript in which the carboxy terminus is truncated, resulting in a 742-amino acid protein that cannot bind GCs and regulates a set of genes distinct from those regulated by GRα. When co-expressed with GRα, GRβ acts as a dominant negative, and has been implicated in GC resistance[21]. Many other GR isoforms can be produced by alternative translation initiation sites, and these can have different transcriptional, transactivation and transrepression profiles. GRα is broadly expressed, but the other isoforms are expressed in specific tissue or tumour cell types and contribute to cell-specific GC responses[22,23].

The unliganded GR resides in the cytosol bound with HSP90, HSP70 and immunophilins. These chaperones maintain the ligand-binding site in an open conformation available for GC binding and prevent nuclear translocation[24]. Ligand binding causes dissociation of this multiprotein complex, GR translocation to the nucleus and GR binding to specific DNA sequences within regions of open chromatin[25] as dimers or tetramers[26]. Transcriptional activation is driven by binding to canonical GC response elements (consensus sequence AGAACAnnnTGTTCT), which causes conformational changes in the GR transactivation

domains that allow recruitment of co-regulators and other transcription factors, driving gene transcription[27]. The GR also binds to negative GC response elements (consensus sequence CTCC(n)$_{0–2}$GGAGA) that directly repress gene transcription[28], and composite sites at which a GR binding sequence is directly adjacent to that of another interacting transcription factor[29,30]. The liganded GR also associates with other transcription factors and inhibits their activity; this may occur independently of GR DNA binding (tethering)[31,32] or involve GR binding to cryptic sites (such as AATTY) within other transcription factor response elements[33,34]. In addition to these genomic mechanisms, GCs can act via non-genomic pathways. For example, ligand binding of membrane-associated GR can initiate signalling via MAPK and PI3K phosphorylation cascades[35,36]. Together, these genomic and non-genomic pathways allow GR to regulate expression of a huge number of genes, perhaps 20% of expressed genes in a given cell type[37]. Cell-specific regulation of GR activity is further determined by post-translational modifications and co-expressed co-regulator proteins. Together, these factors result in highly heterogeneous tissue-specific and cell-specific GC responses[38].

Glucocorticoids and T cell development

T cell development occurs in the thymus: bone marrow-derived lymphoid progenitors enter the thymus, undergo T cell lineage commitment and initiate a series of genomic rearrangements that result in expression of the TCR. TCRs are composed of two variable chains that form a combining site capable of recognizing specific ligands (typically, peptides complexed with MHC-encoded molecules) and the invariant CD3 and ζ-signalling chains. The best characterized of these receptors express α-chains and β-chains, which for practical purposes are unique in every T cell, resulting in a diverse antigen-specific repertoire that allows detection of virtually any peptide antigen capable of binding MHC. Randomly generated TCRs are tested for the ability to respond to self peptide–MHC (pMHC), first in CD4$^+$CD8$^+$ double-positive cells in the thymic cortex and then in CD4$^+$CD8$^-$ and CD4$^-$CD8$^+$ single-positive cells in the medulla. Cortical double-positive thymocytes bearing TCRs with insufficient affinity for self pMHC die by 'neglect', those with sufficient affinity for self pMHC survive and begin the transition to the single-positive stage and move to the medulla (positive selection), and thymocytes with too high an affinity for self pMHC undergo apoptosis (negative selection). In this way, thymic selection ensures the generation of a self-tolerant but effective TCR repertoire.

Thymic homeostasis. All thymocytes express the GR, but levels change during development: GR protein is highest in CD4$^-$CD8$^-$ double-negative cells, lowest in double-positive cells and intermediate in single-positive cells[39,40]. Paradoxically, despite expressing the lowest levels of GR, double-positive thymocytes are the most sensitive to GC-induced apoptosis[41,42], which proceeds via the intrinsic (mitochondrial) pathway through expression of the BH3-only proteins BIM and PUMA[43,44] and activation of BAK and BAX[45]. The initial characterization of the stress response described an acute reduction in thymus size[46], and virtually any stimulus inducing adrenal GC production, including psychological stress[47,48], fasting[49], injury[49] and infection[41,50], can rapidly cause thymic involution of as much as 80% or more. As double-positive thymocytes are constantly replenished by rapidly proliferating precursors, the thymus recovers rapidly after GC basal levels are restored[48]. Thus, thymic involution closely and dynamically follows changes in systemic GC levels.

Death by neglect. At least 85% of double-positive thymocytes undergo death by neglect[51]. Their extreme sensitivity to GC-induced apoptosis and approximately twofold increase in number after adrenalectomy originally led to the assumption that death by neglect is driven by GCs. However, although death by neglect does occur via the intrinsic apoptosis pathway[52], the evidence indicates that basal GCs are not responsible for this process. First, death by neglect involves sensitization to pro-apoptotic BIM via loss of anti-apoptotic BCL-X$_L$ rather than the upregulation of BIM or PUMA[53,54] that is necessary for GC-induced apoptosis of double-positive thymocytes[44]. Second, mice with GR-deficient thymocytes do not have an increase in the number of double-positive thymocytes or appear to acquire new TCR specificities as would be expected in the absence of death by neglect due to the survival of thymocytes with normally subthreshold-signalling TCRs[55]; in fact, double-positive and single-positive thymocyte numbers are actually reduced rather than expanded. Last, adrenalectomy increases double-positive thymocyte numbers even in mice with GR-deficient thymocytes, showing that systemic upregulation of ACTH (due to loss of GC-mediated negative feedback) rather than loss of systemic GCs drives the increase in double-positive cells[56]. Thus, endogenous GR signalling does not appear to be involved in the elimination of 'useless' thymocytes.

Negative selection. Negative selection, the outcome for perhaps 8% of double-positive thymocytes[51] (although estimates have varied widely[57]), occurs via upregulation of BIM and PUMA in response to strong TCR signalling[58,59]. How TCR signalling induces expression of these BH3-only proteins is unclear, but at least in part depends on the pro-apoptotic TCR-induced genes *Nr4a1* (encoding Nur77) and *Ikzf2* (encoding Helios)[60,61]. The first indication that GCs might participate in antigen-specific selection came from studies with T cell hybridomas in which GCs and TCR signalling, each individually pro-apoptotic, together promoted survival (mutual antagonism)[62]. Confirmation of this phenomenon in primary thymocytes[63,64] raised the possibility that GCs counter moderate-affinity TCR signals and allow survival (positive selection) of T cells that would otherwise undergo apoptosis (negative selection). Strong TCR signalling would tip the balance towards negative selection.

Investigation into the role of endogenous GCs in thymocyte development initially led to conflicting

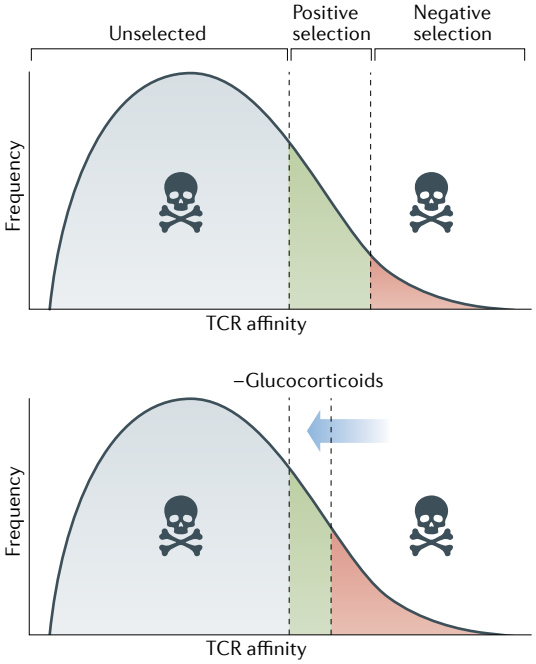

Fig. 2 | **Glucocorticoids antagonize negative selection.** Schematic illustrating the relationship between T cell receptor (TCR) affinity for self peptide–MHC (pMHC) and cell fate. A reduction in glucocorticoid levels or in thymocyte responsiveness to glucocorticoids would shift the boundary between positive and negative selection to the left, resulting in the elimination of thymocytes that otherwise would have been positively selected, resulting in an altered TCR repertoire.

results. Mice expressing a proximal *Lck*-driven GR antisense transgene had increased negative selection of thymocytes[65] and 'holes' in the mature TCR repertoire[66], whereas embryonic thymuses from mice deficient in GR exon 2 had no alterations in thymocyte numbers, proportions or responses to in vitro TCR stimulation[67]. This controversy[68] was resolved by the discovery that deletion of exon 2 results in the production of a ligand-responsive GR fragment still capable of binding DNA and altering gene expression in response to GCs[69]. The role of endogenous GCs in thymocyte selection has now been shown in multiple mouse models with thymocyte-specific deletion of GR exon 3, which results in true GR knockout and complete loss of GR function. These mice have normal or reduced double-positive and single-positive thymocyte numbers[55,66,70–72], an altered TCR repertoire[55,66] and weakened T cell-dependent responses to alloantigen[55], peptide immunization[55,71,72] and viral[55] or bacterial[71] infection, indicating an overall weakening of the TCR repertoire (FIG. 2). Direct evidence for this comes from the finding that, unlike polyclonal T cells, GR-deficient T cells that express a transgenic antigen-specific αβ TCR respond normally to antigen, demonstrating that it is the effector TCR repertoire that is altered by GC-insensitivity during development[55]. Although the proportion of $CD4^+FOXP3^+$ regulatory T (T_{reg}) cells is unaltered in mice with thymocyte-specific GR deletion, whether the T_{reg} cell repertoire is similarly altered by GCs is unknown.

One difficulty in understanding how GCs could regulate an ongoing process such as selection is the extreme variation that occurs in circulating GC levels during early ontogeny and adult life[73–75]. A possible solution was provided by the identification of the thymus as the first non-adrenal organ capable of de novo GC synthesis[14], in which thymic epithelial cells (TECs) express the full suite of GC-synthetic enzymes and generate GCs when cultured in vitro[14,76,77]. Although no de novo GC production by thymocytes was detected in those studies, others reported that cultured thymocytes produced GCs[78,79] and proposed that they are an additional source of de novo synthesized product. However, further examination found that, unlike TECs, thymocytes lack expression and enzymatic activity of the GC-synthetic enzyme Cyp11b1, but express high levels of 11β-HSD1 and regenerate GCs from inactive metabolites[18,80]. Thus, de novo thymus GC synthesis is due to Cyp11b1 expression in TECs and not thymocytes. Autonomous GC synthesis by TECs means that even in the absence of circulating GCs during early development[74], the thymus is able to maintain local GC levels[75,81]. The importance of TEC-derived or thymocyte-derived GCs was tested in conditional knockout mice with TEC-specific or thymocyte-specific deletion of Cyp11b1 ($Cyp11b1^{foxn1\text{-}Cre}$ or $Cyp11b1^{lck\text{-}Cre}$, respectively), ablating the ability to synthesize GCs de novo. In unstressed $Cyp11b1^{foxn1\text{-}Cre}$ mice, total thymocyte expression of GC-induced genes was reduced to the same extent as in mice with GR-deficient thymocytes ($GR^{lck\text{-}Cre}$) or global Cyp11b1-deficiency ($Cyp11b1^{actin\text{-}Cre}$), indicating that TECs are the major source of GCs in the thymus even in adrenal-intact mice[13]. Additionally, $Cyp11b1^{foxn1\text{-}Cre}$ thymocytes had increased markers of negative selection (PD1, Helios, BIM), apoptosis and weakened T cell responses to infection, as is seen in $GR^{lck\text{-}Cre}$ mice. GC-responsive genes and thymocyte selection were unaltered in $Cyp11b1^{lck\text{-}Cre}$ mice, confirming the lack of de novo synthesis in thymocytes[13]. Interestingly, loss of TEC GC synthesis results in diminished GR–chromatin interactions only in antigen-signalled double-positive thymocytes (those that are undergoing selection), and it was estimated that double-positive TCR^{hi} cells are exposed to an approximately threefold higher concentration of GC than other thymocytes in the wild-type thymus. As double-positive TCR^{hi} thymocytes make up a very small proportion of the total (<5%), this illustrates the highly targeted nature of paracrine GC signalling (FIG. 3)[82].

The GC-regulated genes involved in antagonism of negative selection have begun to be characterized. Direct association and mutual inhibition between the liganded GR and the pro-apoptotic orphan nuclear receptor Nur77 (REF.[83]) is one likely mechanism, because the enhanced negative selection of single-positive GR-deficient thymocytes was rescued by loss of Nur77 (REF.[84]). Additionally, GCs inhibit thymocyte upregulation of Helios, and the increased negative selection of double-positive and single-positive GR-deficient thymocytes is completely rescued by the additional deletion of Helios[84]. These GR actions may involve its co-regulator NCOR1, which has been independently shown to promote positive selection[85].

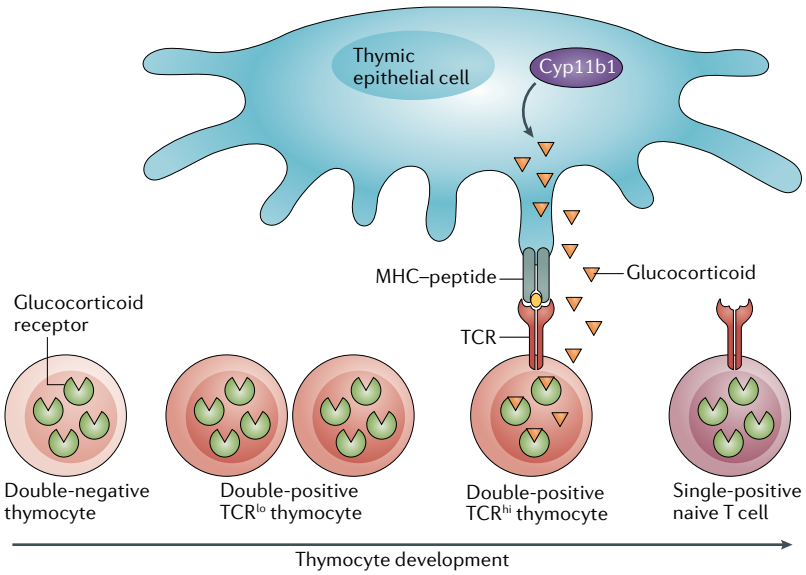

Fig. 3 | **Paracrine glucocorticoid signalling in the thymus.** Thymic epithelial cell-synthesized glucocorticoids are specifically delivered to antigen-signalled double-positive thymocytes ($CD4^+CD8^+TCR^{hi}$), resulting in decreased expression of proteins that promote negative selection, such as Helios, PD1 and BIM, and thus promoting positive selection. TCR, T cell receptor.

Exposure to elevated glucocorticoids in early life. Fetal and childhood exposure to elevated GC levels, when thymopoiesis is most active, is very common whether in response to stressors or exogenous administration. Elevated GC exposure during development has been shown to alter the risk of immune-mediated disease in later life[86], and the effects of GCs on thymocyte development might contribute to long-term effects on T cell-mediated immune responses. One mechanism may be through alterations in the TCR repertoire. In mice, a single prenatal GC treatment briefly decreases the thymus size[87] but causes lasting alterations in the peripheral TCR repertoire, decreases the incidence of diabetes in NOD mice and increases autoreactivity in autoimmune-prone MRL/lpr mice[88]. Chronic exposure to elevated GC levels during development can have long-term programming effects on the hypothalamus–pituitary–adrenal axis, altering basal and stressed GC secretion[89] and, in turn, GC homeostatic regulation of T cell function. For example, GC administration for 2 weeks during mouse fetal development permanently reduces basal and stress-induced adrenal GC secretion, resulting in stable alterations in T cell chromatin accessibility, a metabolic shift to oxidative phosphorylation and reduced effector function[72]. Such GC-treated mice have reduced $CD4^+$ and $CD8^+$ T cell responses to immunization and increased syngeneic and allogeneic tumour growth, and show reduced clearance of the bacterial pathogen *Listeria monocytogenes*. These observations suggest that environmentally stimulated alterations in endogenous GC production might have pronounced effects on the T cell repertoire and on the ability to respond to various immune challenges throughout life. It is interesting to speculate whether such phenotypic plasticity serves an evolutionarily adaptive function, perhaps for individuals raised in difficult (for example, high stress) environments to shift towards pathogen tolerance[90].

Glucocorticoids and T cell function

Immunosuppression by glucocorticoids. Arguably, the best known effect of GCs is their powerful ability to suppress the immune response. Supraphysiological doses of exogenous GCs such as betamethasone, dexamethasone and prednisone are a mainstay of therapies aimed at reducing autoimmunity, transplant rejection and inflammation, and our understanding of GC effects on immunity has, unsurprisingly, derived primarily from studies using high doses of synthetic GCs such as dexamethasone and prednisone. The resulting conclusion is that GCs are universally immunosuppressive and even lymphotoxic. Endogenous GCs, in contrast to this view, are now understood to have diverse enhancing effects on T cell function, which are discussed below. Nonetheless, by far the most potent effect of GCs on immune function, and on T cells in particular, is that of immunosuppression.

Immune activation and the resulting increase in circulating levels of pro-inflammatory cytokines is one of the most potent inducers of adrenal GC synthesis. Systemic GC levels rise rapidly in response to a broad range of pathogens, including many viral[50,91], bacterial[72] and parasitic[41,92] infections. Adrenal GC production closely parallels increases in cytokines such as IFNγ, IL-1β, IL-6 and TNF, and can begin within hours of infection, often in response to production of these cytokines by innate immune cells[93]. T cell-derived cytokines (including IL-6, IL-8, IFNγ, GM-CSF and TNF) further stimulate adrenal GC synthesis[41,70,92]. Whereas GC signalling can have enhancing effects on T cell function (see below), the primary effect of elevated GCs is overwhelmingly suppression of T cell effector responses. In the absence of adrenal GC production, pathogen clearance can be much more rapid[41,50,91] but comes at the cost of increased mortality due to uninhibited T cell responses, cytokine storm and vascular shock[41,50,70,92]. Thus, endogenous GCs are a vital and non-redundant brake on effector T cell responses.

GCs can suppress the initiation of T cell responses by reducing the antigen presentation, co-stimulation and cytokine production functions of innate immune cells[94–97]. Many of the most important effects of GCs, however, are their direct actions upon T cells, largely via regulation of transcription: increased expression of immunoregulatory proteins, inhibitory receptors and apoptotic genes, and decreased expression of pro-inflammatory cytokines, co-stimulatory molecules and cell cycle mediators. Liganded GRs induce transcription of immunosuppressive genes such as *Tsc22d3*, *Dusp1*, and *Nfkbia* (which encode GILZ, MKP1 and IκBα, respectively)[98,99]. GILZ (glucocorticoid-induced leucine zipper) associates with NF-κB and AP-1 to prevent their transactivation of inflammatory and cytokine genes[100,101] and associates with RAS and RAF to prevent induction of AKT-induced and ERK-induced proliferation[102]. MKP1 is a dual-specificity phosphatase that inactivates ERK, JNK and p38, and thus inhibits cytokine expression and cell proliferation[103], and IκBα

sequesters NF-κB in the cytosol to inhibit its upregulation of numerous cytokines. The GR also directly associates with NF-κB, AP-1 and Nur77 family proteins to inhibit their transcriptional activity[32,83,104,105]. The result is dramatic: GCs suppress T cell expression of co-stimulatory molecules (such as CD2, CD28 and 41BB)[37], cytokines (including IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-9, IL-13, IL-22, TNF, TSLP, IFNα, IFNβ and IFNγ[28,37,70,98,106–112]) and chemokines (including CCL3, CCL4, CCL5, CCL7, CCL8, CCL11 and CCL13 (REFS[37,113,114])). Furthermore, GCs upregulate co-inhibitory molecules such as PD1, CTLA4, LAG3 and TIM3 (REFS[37,115–117]). The overall result is powerful suppression of T cell effector programmes.

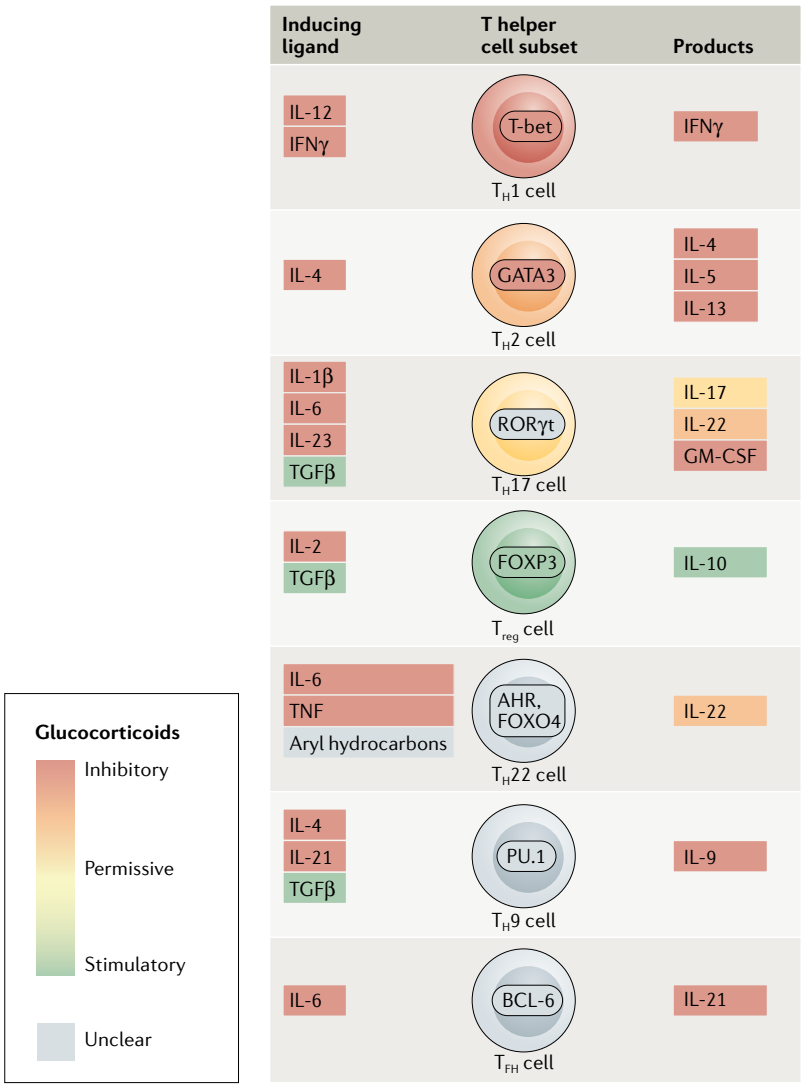

Fig. 4 | **GC regulation of effector CD4⁺ T cell differentiation.** Glucocorticoids (GCs) control and shape T cell responses by differentially regulating the inducing signals, master transcription factors and effector cytokines of CD4⁺ T helper cells. The figure indicates whether GCs have an inhibitory, permissive, stimulatory or unclear effect on key factors associated with type 1 T helper (T_H1) cell, T_H2 cell, IL-9-producing T helper (T_H9) cell, T_H17 cell, T_H22 cell, CD4⁺FOXP3⁺ regulatory T (T_{reg}) cell and T follicular helper (T_{FH}) cell subsets. AHR, aryl hydrocarbon receptor; BCL-6, B cell lymphoma 6; FOXP3, forkhead box P3; GATA3, GATA-binding protein 3; GM-CSF, granulocyte–macrophage colony-stimulating factor; RORγt, RAR-related orphan receptor-γt; TGFβ, transforming growth factor-β; TNF, tumour necrosis factor.

T helper cell differentiation. CD4⁺ T helper cells and CD8⁺ cytotoxic T cells differentiate into various effector phenotypes depending on the cytokine milieu in which their activation takes place (FIG. 4). Whereas these phenotypes fall on a continuum, the recognition of distinct T helper cell subsets has been invaluable in investigating and understanding different types of immune responses. The GR is expressed by all T cells but, as in the thymus, different cells have dramatic differences in GC sensitivity. Thus, although the overall effect of GC signalling is suppression of T cell activation, differential suppression of subsets effectively means that GCs promote particular T helper cell responses over others: they potently suppress inflammatory type 1 T helper (T_H1) cell responses, moderately suppress T_H2 cell responses and are permissive of IL-17-producing T helper (T_H17) cell responses.

Initial T helper cell polarization is dependent on signals from innate immune cells, and GC regulation of cytokine synthesis at this early stage can begin to shape the ultimate T cell response by directing T helper cell differentiation. For example, GCs potently inhibit macrophage and dendritic cell production of IL-12 and IFNγ[95,96], reducing T_H1 cell induction. T cell-derived cytokines, however, maintain and amplify T cell differentiating conditions during ongoing immune responses[118], and direct GC signalling in T cells is in large part responsible for GC effects on T cell differentiation. GCs globally inhibit T_H1 cell responses. Inhibition of IL-12-induced STAT4 phosphorylation prevents its activation and resulting transcriptional activity[119], and inhibition of STAT1 gene expression prevents IFNγ signalling[120], with both effects preventing T_H1 cell differentiation. GCs furthermore inhibit expression of T-bet (*Tbx21*) and IFNγ (*Ifng*) genes, and the GR directly associates with T-bet protein to prevent expression of a T_H1 cell transcriptional programme[108,111,112].

GCs also suppress T_H2 cell differentiation, but to a much lesser extent than T_H1 cell differentiation, which in effect preferentially allows T_H2 cell responses. Inhibition of innate cell IL-12 and IFNγ production, by preventing T_H1 cell differentiation, frees T_H2 cells from inhibition. GCs have little effect on IL-4-induced STAT6 phosphorylation[119], but GC induction of MKP1 inhibits p38 activation and induction of GATA3 (REFS[98,109]), hence preventing expression of IL-4, IL-5 and IL-13 (REFS[106,110,111]).

Finally, GCs upregulate T cell expression of the IL-1 and TGFβ receptors[37] and synergize with IL-6-activated STAT3 (REF.[121]) to promote T_H17 cell differentiation. T_H17 cells are often refractory to GCs[111,112,122], which is at least in part owing to increased expression of the GC-exporting membrane channel MDR1 (encoded by *ABCB1*)[123]. Furthermore, GCs increase RORγt expression[112] and IL-17 production[111,122], although they can suppress IL-22 (REFS[111,112]) and GM-CSF[110]. Generally, therefore, GCs are permissive of and even promote T_H17 responses. Less well studied are T_H9 and T_H22 cells, and little is known about their regulation by GCs other than that the secretion of their signature cytokines — IL-9 and IL-22, respectively — is suppressed by GCs in vitro[107,111,112]. This could be owing to GR suppression of PU.1 activity[124] and AHR expression[125], but this has yet

to be systematically examined in T cells. GC effects on T follicular helper cells are even less clear, as GCs inhibit T cell IL-21 production[126] but also have been shown to upregulate BCL-6 expression in non-T cells[127]. Thus, there is a clear hierarchy of GC effects on T helper cell differentiation, with strong inhibition of T_H1 cells, moderate inhibition of T_H2 cells and permission for T_H17 cell responses. This hierarchy extends to T helper cell survival, with GC treatment causing at least threefold greater specific apoptosis in T_H1 cells compared with that in T_H2 cell and T_H17 cell cultures. This difference is due to GC-induced loss of BCL-2 and greater induction of BIM in T_H1 cells, with stable or increased BCL-2 expression in T_H2 cells and T_H17 cells[111].

Unlike T helper cells, extrathymic T_{reg} cell differentiation is clearly promoted by GC signalling. Upregulation of TGFβ receptors[37], FOXP3 (REFS[128,129]) and IL-10 (REFS[37,112]) is consistent with increased T_{reg} cell differentiation and function. Furthermore, the GR is upregulated during T_{reg} cell differentiation[129] and GC-responsive *Gilz* promotes T_{reg} cell differentiation[130]. In fact, although transgenic overexpression of GR in T cells reduced T helper cell numbers by approximately half, it had no suppressive effect on, and may even have increased, T_{reg} cell numbers, as these mice were found to have increased proportions of T_{reg} cells compared with wild-type mice[116]. In addition, T_{reg} cell-specific loss of GR ($GR^{foxp3\text{-}Cre}$ mice) was shown to exacerbate colitis[131]. Even more dramatically, $GR^{foxp3\text{-}Cre}$ mice are completely refractory to GC treatment of experimental autoimmune encephalitis and cockroach antigen-induced airway allergic reactions, leading to the surprising conclusion that induction of T_{reg} cell activity may be the dominant mechanism of GC immunosuppression during an effector T cell response[132]. Together with greater resistance to GC-induced apoptosis compared with other T helper cells[133,134], these data indicate that GCs play an important role in T_{reg} cell differentiation and function, and support the possibility that enhancement of T_{reg} cell function is a major mechanism by which endogenous GCs effect immunosuppression.

Memory T cell differentiation. Resolution of T cell responses involves the contraction of effector T cell populations and maintenance of memory T cell populations. Memory T cells are derived from precursors that arise at the peak of the effector T cell response and slowly acquire memory T cell characteristics, including the capacity for self-renewal and rapid recall responses to antigen, over the following weeks and months[135]. GC signalling plays an important role in the selection of T cell clones that become memory T cells, and in the differentiation of memory T cells during a response. Perhaps counterintuitively, GC suppression of effector T cell proliferation may be important in the generation of efficient T cell memory. During the effector response, GC signalling selectively inhibits proliferation of T cells with low-affinity but not high-affinity TCRs, and the latter are preferentially recruited into the memory precursor cell pool[136]. This effect is in part due to GC inhibition of fatty acid oxidation, which is critical for memory T cell survival. This GC function is reminiscent of that in the thymus, where GC antagonism of TCR signalling in effect promotes survival of a more strongly reactive TCR repertoire. However, unlike the thymus, this results in the loss of weakly reactive TCR clones, reducing the diversity of responding TCRs upon subsequent exposure. As there is obviously a limited niche for memory T cells, such selection may ensure that only T cells with high-affinity TCRs survive, optimizing the memory T cell pool.

In addition to affecting which T cells expand during an immune response, GC signalling is also critical in the differentiation of memory T cells. $CD8^+$ memory precursor cells express low levels of KLRG1, a molecule preferentially expressed by terminal effector T cells, and high levels IL-7Rα (CD127), which is low in terminal effector cells[137]. IL-7 signalling via IL-7Rα induces phosphorylation of STAT5, which in turn upregulates expression of anti-apoptotic BCL-2 and BCL-X_L, which are instrumental in long-term memory T cell survival[137]. The finding that GCs upregulate T cell expression of *Il7ra* (which encodes IL-7Rα)[138,139] suggested a mechanism by which they might enhance T cell memory. GC signalling has recently been directly implicated in memory T cell differentiation, with epigenetic and transcriptomic surveys identifying *Nr3c1* (encoding the GR) as a central driver of the memory T cell transcriptional programme, with upregulation of GC-induced genes such as *Il7ra*, *Cxcr4*, *Tgfbr1*, *Tgfbr2* and *Foxp1*. Short hairpin RNA-mediated knockdown of GR in mature T cells adoptively transferred into naive recipients that were subsequently infected with *L. monocytogenes* shifted differentiation towards a terminal effector programme instead of a memory precursor cell programme, with a dramatic decrease in the number and frequency of memory precursor ($IL\text{-}7R\alpha^+KLRG1^-$) cells at the peak of the reponse[140]. Deletion of the GR co-activator *Ncor1* gave the same result, further implicating GR transcriptional activity as a central component of memory precursor differentiation[140]. GR–IL-7Rα-mediated induction of BCL-2 is also necessary for long-term maintenance of memory T cells, as the memory cell number is markedly reduced in the absence of T cell GR expression[71,140]. Rather than solely suppressing T cell responses, therefore, GC signalling selectively suppresses responses and differentiation of effector cells to promote the generation of memory cells.

Immune enhancement. A growing body of work demonstrates that endogenous GC signalling in T cells also plays an important positive preparative role before and during the early stages of immune responses. Primary among these may be GC induction of the chemokine receptor CXCR4, which promotes trafficking to $CXCL12^+$ lymphoid organs. Adrenal GC secretion is controlled by the central circadian clock in the suprachiasmatic nucleus of the hypothalamus, and functions in part to entrain cyclic brain and metabolic activity. In diurnal humans, circulating GC levels peak at dawn and reach their nadir at dusk, and in nocturnal mice they peak at dusk and reach their lowest levels at dawn. Given the exquisite sensitivity of T cells to GCs, it would be remarkable if circadian GC fluctuations did not affect

T cell function, and studies in humans and mice have discovered that lymphocyte trafficking between compartments indeed follows a circadian cycle[71,141]. The distribution of T helper cells, cytotoxic T cells and T_{reg} cells between the blood and secondary lymphoid organs fluctuates during the day. In mice, the blood to lymphoid organ ratio reaches a peak early during the sleep period (4 h after lights on) and a nadir early during the waking period (4 h after lights off), meaning that T cells favour a lymphoid locale during the active period[71]. This trafficking between the blood and lymphoid organs is directly regulated by GCs via upregulation of IL-7Rα and, subsequently, the lymphoid-homing chemokine receptor CXCR4 (FIG. 5). The same GC–CXCR4 pattern drives circadian T cell redistribution in humans[141] and, in the absence of GR signalling, IL-7Rα or CXCR4, T cell numbers remain high in the blood and low in lymphoid organs[71,141]. Remarkably, this means that the diurnal timing of an immune challenge has dramatic effects on the response. For example, the week after infection of mice with *L. monocytogenes*, the numbers and proportions of pathogen-specific $CD8^+$ T cells were at least twice as high when infection occurred during the active (dark) period rather than the sleep (light) period. Similar results occur with T follicular helper cell and germinal centre B cell responses to immunization, and in both cases this circadian difference was lost if T cells were unresponsive to GCs[71]. It is attractive to hypothesize that this cycle evolved to allow the limited number of pathogen-specific T cells to reside in lymphoid tissues, where antigen exposure and initiation of B cell germinal centre responses occurs, during the active period when an encounter with such hazards is most likely. Temporally sequestering these activities seems intuitively adaptive — trafficking during the sleep cycle, when the probability of injury and exposure to infectious agents may be reduced, and maximizing T cell availability in lymphoid tissues in the active period, when the probability of exposure is increased.

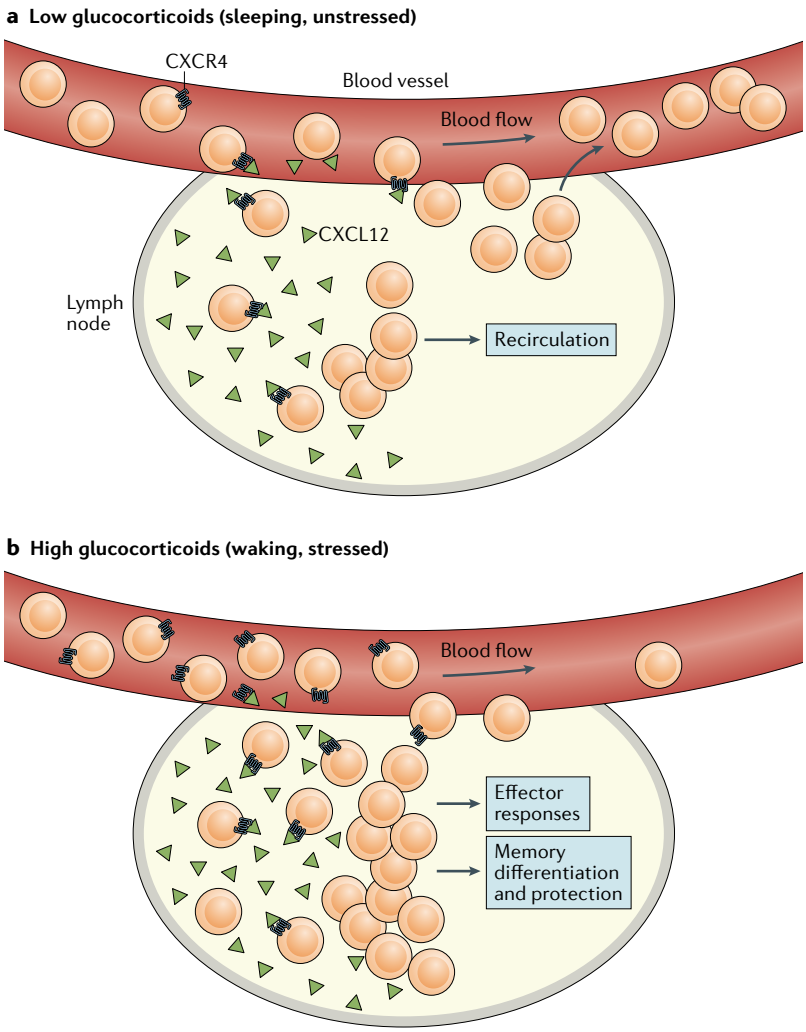

Fig. 5 | **Circulating glucocorticoids regulate T cell trafficking and resulting effector and memory responses.** Glucocorticoids upregulate T cell expression of the IL-7 receptor, whose signalling in turn upregulates expression of C-X-C chemokine receptor type 4 (CXCR4). Expression of CXCR4 results in T cell homing to lymphoid organs that express its ligand, C-X-C motif chemokine 12 (CXCL12). **a** | When glucocorticoid levels are low, such as during the sleep period or unstressed conditions, T cells express little CXCR4 and their numbers are enriched in the blood and reduced in lymphoid organs. **b** | During the onset of the waking period or during stress, increased glucocorticoid levels upregulate T cell expression of CXCR4 and induce trafficking into lymphoid tissues. Glucocorticoid-induced CXCR4 upregulation thus promotes effector responses and differentiation of memory cells.

GC fluctuations in response to acute stressors are more pronounced than those occurring over the circadian cycle, and have long been known to reduce the number of lymphocytes in the blood. This was originally attributed to the lympholytic effects of GC[142], but further investigation prompted by the idea that massive apoptosis would be an immensely maladaptive physiological response led to the discovery that GC-induced lymphopenia was due to redistribution from the blood to lymphoid tissues[143,144]. Acute stress (minutes/hours) indeed results in greater trafficking of innate cells and lymphocytes to lymphoid organs and barrier tissues, and enhances dendritic cell migration to lymph nodes[145]. Like circadian changes in distribution, this trafficking of T cells is via GC induction of CXCR4 expression[146] and homing to $CXCL12^+$ lymphoid organs[147]. This enhances subsequent T cell-mediated delayed-type hypersensitivity responses to exogenous antigens[148] and improves recall responses upon re-exposure, including a greater number of responding T cells and increased production of pro-inflammatory cytokines[149]. Prolonged stress (days or longer) also results in induction of CXCR4 and T cell redistribution. Instead of priming T cell responsiveness, however, this appears to play more of a protective role. Dietary restriction, an effective inducer of GC synthesis, reduces T cell numbers in secondary lymphoid organs but increases numbers in the $CXCR12^+$ bone marrow[150]. Bone marrow accumulation of memory T cells is especially pronounced, and upon entering the marrow these cells assume a quiescent state. Trafficking to the bone marrow during dietary restriction allows memory T cells to better mount a response upon re-exposure to a previously cleared pathogen, indicating that the bone marrow serves as a protective niche for maintenance of the memory T cell pool during periods of prolonged stress, such as in the presence of insufficient energy resources[150].

Conclusions

The immunosuppressive effects of GCs on T cells are well known, including their inhibition of proliferation and cytokine production and induction of apoptosis in T cells. Recently, however, it has become clear that, in addition to preventing lethal overshoot of the immune response, the suppressive actions of endogenous GCs provide a counterbalance with graded effects on different aspects of T cell activation, which in turn shapes T cell development and differentiation. In the thymus, GC signalling selectively targets pMHC-stimulated double-positive thymocytes, promoting positive selection and increasing the overall strength of the TCR repertoire, whereas paracrine GC signalling at barrier sites provides local immunosuppression while avoiding systemic effects. During T helper cell differentiation, variations in susceptibility to the suppressive effects of GCs shape the quality of the response, permitting T_H17 cell responses while strongly inhibiting T_H1 cell responses, with intermediate effects on T_H2 cells. Furthermore, inhibition of weakly signalled effector T cells prevents their incorporation into the memory T cell pool. Across contexts, therefore, suppression by GCs acts as a selective force that, by paring, actually optimizes T cell responses. In parallel, it is clear that GC induction of GR–IL-7Rα–CXCR4 signalling has enhancing effects on T cell immunity. By appropriately timing T cell trafficking through the bloodstream and lymphoid organs, this signal cascade primes the ability of T cells to respond to pathogens, an early effect that is amplified over the course of the immune response. This same signal cascade subsequently drives the differentiation and survival of memory T cells, ensuring their ability to respond efficiently upon pathogen re-exposure. In summary, endogenous GCs can act as both a brake and a driver of T cell responses, and as such are critical for immunological fitness.

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