– follicular regulatory T (Tfr) cells form after immunisation and are able to access the GC, where they control the size and output of the response. Our knowledge lls -8.669 13.222 -7.23 13.891 -6.125 13.891 c -4.853 13.891 -4.385 13.255 -

zone that contains FDC bearing immune complexes and follicular helper T (Tfh) cells. B cells with somatically mutated BCRs collect antigen from the surface of FDC, internalise it and present it to Tfh cells in the context of major histocompatibility complex class II (MHC-II). B cells with the highest affinity BCRs are able to outcompete lower affinity B cells for T-cell help, resulting in further clonal expansion of high-affinity GC B cells and formation of high-affinity plasma cells and memory B cells [14,15]. This process of mutation and selection that generates effector B cells with BCRs with increased affinity for antigen is referred to as affinity maturation, and competition for Tfh cell help is an essential mediator of this [15].

Follicular helper T cells

Tfh cells are essential for the formation and maintenance of the GC response [16]. Tfh differentiation is initiated by priming of the $CD4^+$ T cell by dendritic cells (DCs) via the engagement of the T-cell receptor (TCR) by the MHC-II peptide complex on DCs in conjunction with co-stimulation between CD80/CD86 on the DC and CD28 on the T cell. During these T:DC interactions, the cytokines IL-6 and IL-12 and the co-stimulatory molecule inducible co-stimulator (ICOS) support differentiation into Tfh precursor cells [17]. These signals are critical for induction of the transcription factor B-cell lymphoma (Bcl)-6 [18], which is both necessary and sufficient for Tfh differentiation [19-21]. Bcl-6 promotes Tfh differentiation by actively repressing the Th1 (Tbet), Th2 (GATA-binding-protein 3 (GATA3)), Th17 (retinoid-orphan receptor gamma ($ROR\gamma t$)) and regulatory T (Treg) (forkhead box p3 (Foxp3)) master transcription factors as well as the transcription factor B-lymphocyte-induced maturation protein 1 (Blimp-1) [19-21]. Bcl-6 and Blimp-1 are mutually antagonistic and the balance of these two transcription factors is essential for optimal Tfh cell differentiation [21].

Expression of CXCR5 allows T cells to migrate to the T:B border towards the ligand for CXCR5, CXCL13, which is expressed by FDC in the follicle [22,23]. Induction of CXCR5 on Tfh precursor cells is mediated by the expression of the transcription factor achaete-scute complex homolog 2 (Ascl-2) [24]. After T-cell priming, Tfh precursor cells need to interact with B cells in order to fully differentiate into GC Tfh cells; these stable interactions between Tfh precursor cells and B cells are mediated by ligation of the SLAM family of receptors, CD84 and Ly108, supported by the intracellular adaptor molecule SLAM-associated protein (SAP) [25]. Tfh cells also receive signals through ICOS during interactions with bystander B cells; this induces the phosphatidylinositide 3-kinase pathway and triggers actin waves and polarised pseudopod formation, facilitating the migration of Tfh cells to the follicle [26]. Once within the GC, Tfh cells provide help to GC B cells.

Fully differentiated GC Tfh cells provide survival and differentiation signals to GC B cells through CD40 ligand–CD40 interactions and secretion of cytokines, including IL-21 and IL-4 [27-31]. GC B cells compete with each other for help from Tfh cells by presenting antigen on MHC-II. Those GC B cells that can engage Tfh cells will receive survival and differentiation cues to exit the GC as long-lived plasma cells or memory cells or to undergo further rounds of somatic hypermutation. The B cells with the highest affinity BCRs are able to collect and then present the most antigen to Tfh cells, thereby outcompeting the lower affinity B cells for T-cell help.

The dialogue between Tfh and GC B cells is not one directional, with B cells providing signals to Tfh cells that control their maintenance and function. PD-1 ligation on Tfh cells during interaction with B cells controls or memory PI

which include spontaneous GC formation and Tfh cell expansion in the absence of immunisation or infection [42-44]. Mice in which Foxp3$^+$ cells lack interferon regulatory factor (IRF)4 develop spontaneous GCs but are not a phenocopy of Foxp3-deficient mice [45]. This observation suggests that IRF4 controls an aspect of Treg biology that is essential for moderating the GC itself and that specific, cell-intrinsic molecular pathways mediate the suppression of the spontaneous GC response by Treg cells. IRF4 is required for expression of the homing molecules CD62L and CD103 and the inhibitory molecule cytotoxic T-lymphocyte antigen 4 (CTLA-4), and changes in these molecules may have implications for Treg control of the GC. There is evidence that CTLA-4 is also a critical

including the expression of several Tfh cell markers such as Tfh lineage-specific transcription factor Bcl-6 along with CXCR5, PD-1, ICOS, and SAP [44,53]. Tfr cells do not express the B-cell helper molecules IL-21, IL-4 and CD40 ligand that are characteristic of Tfh cells [53]. Instead they express a range of typical Treg cell markers in addition to Foxp3, such as glucocorticoid-induced tumour necrosis factor receptor-related protein and CTLA-4 [44,53]. Gene expression profile analysis further showed that Tfr cells have a distinct transcriptional profile that has more in common with Treg cells than Tfh or other Th cells [53].

Because Tfr cells have phenotypic characteristics of both Tfh and Treg cells, it seemed likely that these cells arise from one or the other of these cell types. Thus, Tfr cells could derive from $Foxp3^+$ Treg cells that enter the GC or from Tfh cells that switch on Foxp3 within the GC. To determine the origin of Tfr cells, naïve $Foxp3^-$ TCR-transgenic T cells were transferred into intact re-

has yet to be defined. They could act directly on B cells or Tfh cells through cell–cell interaction, via cytokine production, or through a combination of these. Treg cells can directly inhibit B-cell antibody production in vitro in a cell contact-dependent manner [60]. Tfr cells cannot form in the absence of B cells or SAP, suggesting that Tfr cells or Tfr precursor cells interact with B cells *in vivo* [53]. Thus, Tfr cells may limit the GC response by direct interaction with GC B cells. However, Treg cells are also able to suppress effector T cells directly *in vitro* [52], so Tfr cells may also act by suppressing the expansion or function of Tfh cells through direct contact. One key molecule highly expressed by Treg cells that is implicated in suppression of GC responses is CTLA-4 [61]. When CTLA-4 signalling is blocked during an established GC response, the GC B cells continue to proliferate and GC resolution is prevented. Furthermore, the direct suppression of B cells *in vitro* by Treg cells is partially mediated by CTLA-4 [60]. Thus CTLA-4, in addition to its role in suppressing the initiation of the GC response, may also help in mediating Tfr cell suppression of the GC response.

Tfr cells may also exert their suppressive effects in the GC indirectly via cytokine secretion. One candidate would be IL-10, which has an established role in mediating Treg suppression and is expressed by Tfr cells [16]. Mice with Foxp3^{+} Treg cells deficient in IL-10 do not develop severe autoimmunity but do develop spontaneous gastrointestinal inflammation [62]. The role of IL-10 during immune responses is complex and both IL-10 and its receptor (IL-10R) are broadly expressed by different immune cell types [63]. IL-10 limits the size of the

contributes to autoimmune pathology, and correction of this balance could have therapeutic potential.

Follicular regulatory T cells as a specialised regulatory T-cell subset

In recent years it has become increasingly clear that, like $CD4^+$ Th cells, Treg cells are functionally and phenotypically diverse. $Foxp3^+$ Treg cells can differentiate into several functional states characterised by expression of specific transcription factors classically associated with different Th-cell subsets. For example, during a Th1-skewed response, $Foxp3^+$ Treg cells upregulate Tbet in a STAT1-dependent manner, the key transcription factor for Th1 cell differentiation [71,72]. Tbet expression in Treg cells drives CXCR3 expression, as it does in Th1 cells, and enables these Th1-like Treg cells to migrate towards the site of inflammation where they can specifically regulate Th1 responses. The induction of these $CXCR3^+Tbet^+$ Treg cells occurs in an interferon gamma-dependent manner, similar to Th1 cells [72]. Th17 cell-mediated immune responses can be specifically controlled by Treg cells expressing Th17-associated STAT3, as Treg cell-specific deletion of STAT3 results in fatal intestinal inflammation and excessive Th17 responses [73]. Tfh cells are the critical Th cell population for the GC, and Tfr cells phenotypically resemble Tfh cells in many respects, as described in this