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Signals that drive T follicular helper cell formation

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Summary

T follicular helper (Tfh) cells are a distinct type of $CD4^+$ T cell specialized in providing help to B cells during the germinal centre (GC) reaction. As such, they are critical determinants of the quality of an antibody response following antigen challenge. Excessive production of Tfh cells can result in autoimmunity whereas too few can result in inadequate protection from infection. Hence, their differentiation and maintenance must be tightly regulated to ensure appropriate but limited help to B cells. Unlike the majority of other $CD4^+$ T-cell subsets, Tfh cell differentiation occurs in three phases defined by their anatomical location. During each phase of differentiation the emerging Tfh cells express distinct patterns of co-receptors, which work together with the T-cell receptor (TCR) to drive Tfh differentiation. These signals provided by both TCR and co-receptors during Tfh differentiation alter proliferation, survival, metabolism, cytokine production and transcription factor expression. This review will discuss how engagement of TCR and co-receptors work together to shape the formation and function of Tfh cells.

Keywords: activation; co-stimulation; inhibitory/activating receptors; signal transduction; T follicular helper cell.

Introduction

The induction of long-lasting antibody-mediated immunity depends upon the formation of a productive germinal centre (GC) where B cells can differentiate into memory B cells and antibody-secreting cells.[1,2] During the GC reaction the B cells undergo affinity maturation and antibody class switching. GC formation is dependent upon the ability of T follicular helper (Tfh) cells to interact with B cells and provide them with 'help' in the form of cytokine secretion and co-receptor expression (reviewed in refs 3 and 4). Tfh cells differentiate from naive $CD4^+$ precursors, a process that must be tightly controlled to ensure optimal B-cell help. Too many Tfh cells can lead to autoimmunity whereas too few result in inadequate protection from infection. Hence, Tfh cells are an attractive target for therapeutic intervention. This requires an in-depth understanding of the mechanisms regulating their differentiation and function. A plethora of cytokines, signalling molecules and transcription factors are reported as critical for Tfh cell identity and function. Yet the dominating intracellular signalling pathways that drive Tfh cell differentiation are less well understood. The role of cytokines in driving Tfh cell differentiation has been recently reviewed,[5] so here we will discuss how antigenic and co-receptor signals shape Tfh cell differentiation.

Phases of Tfh cell differentiation

Tfh cell differentiation is a multistage process, occurring over a period of days.[3,4] It can be divided into three phases, broadly defined by the anatomical location of the T cell as illustrated in Fig. 1. In the first phase, naive $CD4^+$ T cells are antigenically stimulated by dendritic cells (DC) in the T-cell zone of secondary lymphoid organs. If the delivery of antigen occurs in combination with delivery of specific cytokine and co-receptor signals,

Abbreviations: Bcl6, B-cell lymphoma 6; CTLA-4, cytotoxic T-lymphocyte antigen 4; DC, dendritic cell; GC, germinal centre; IL-21, interleukin-21; ICOS, inducible T-cell co-stimulator; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor-κB; PD-1, programmed cell death 1; SAP, SLAM-associated protein; SFR, SLAM family receptor; SLAM, signalling lymphocytic activation molecule; TCR, T-cell receptor; Th, T helper; Tfh, T follicular helper

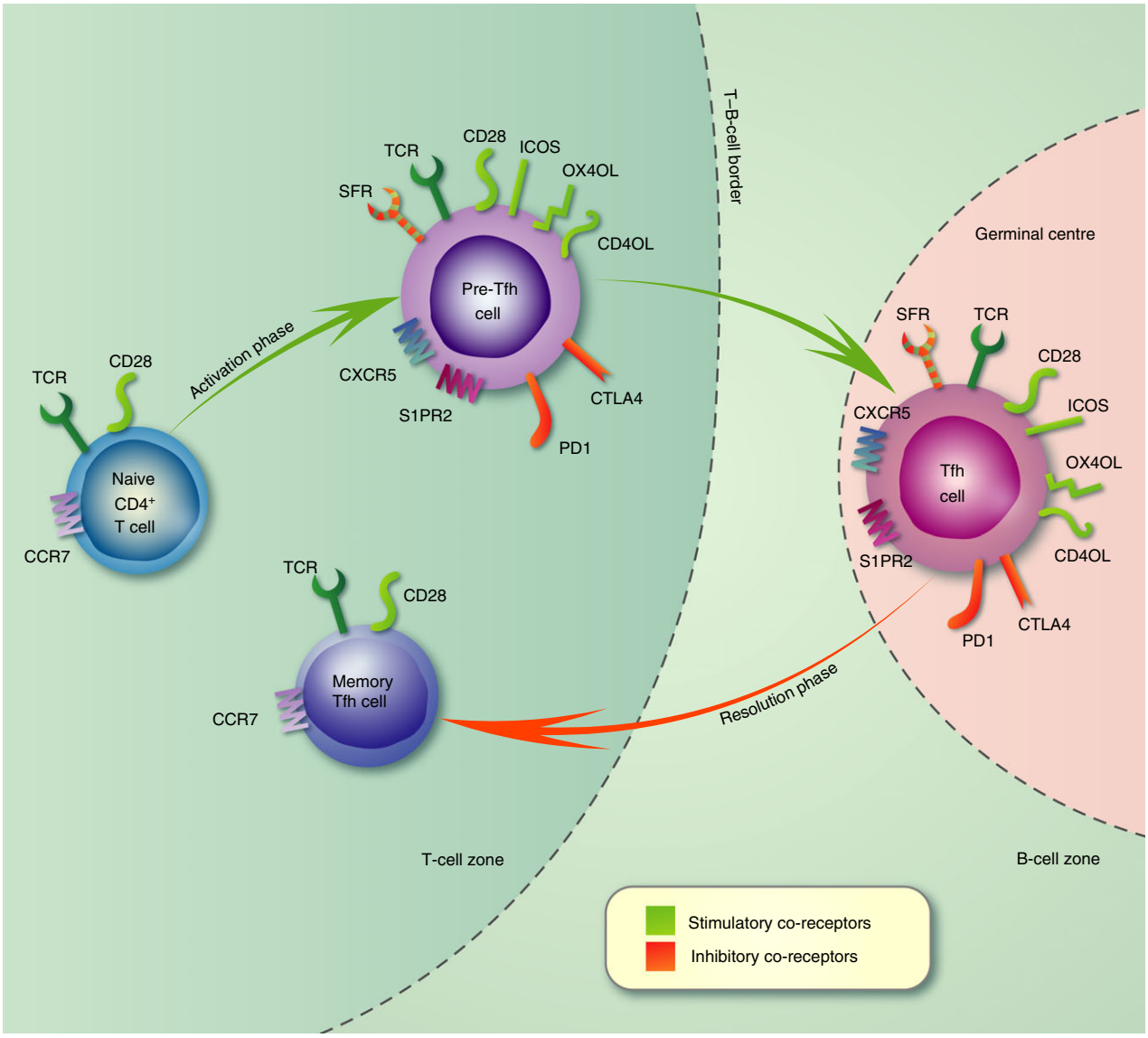

Figure 1. Expression of co-receptors during the three phases of T follicular helper (Tfh) cell differentiation. Schematic depicting the multistage and location-specific nature of Tfh cell differentiation. The expression of co-receptors is shown at each stage with stimulatory receptors coloured green and inhibitory receptors coloured red. During the first phase of differentiation (days 0–3) naive $CD4^+$ T cells are activated by dendritic cells in the T-cell zone where they proliferate and alter expression of co-receptors. This allows the resulting pre-Tfh cell to migrate towards the T–B-cell border where it engages with antigen-specific B cells. Co-receptors expressed by Tfh cells also modulate the signals transduced through the T-cell receptor, allowing their differentiation. In the second phase (days 4–5) antigen is presented by B cells and the co-receptors expressed by pre-Tfh cells are used symbiotically to both induce Tfh cell differentiation and provide co-stimulation to B cells. In the third phase (days 6–10), Tfh cells engage with germinal centre (GC) B cells within the GC. Tfh cells provide a limiting source of help to ensure that appropriate B cells differentiate into antibody-secreting cells and memory B cells. During the resolution phase the Tfh cell can leave the GC and in the absence of further antigenic stimulation resides as a long-lived memory Tfh cell in the periphery.

primed T cells can enter into the Tfh differentiation programme and become pre-Tfh cells that express high levels of programmed cell death protein 1 (PD-1) and inducible T-cell co-stimulator (ICOS). This is accompanied by increased expression of the transcription factors TCF-1, cMAF and B-cell lymphoma 6 (Bcl6), with concurrent repression of Blimp-1. In addition, T cells lose expression of CCR7 and EBI2, and gain expression of CXCR5, CXCR4 and S1PR2 allowing them to respond to chemotactic signals and migrate to the areas of the secondary lymphoid organs where T and B cells meet, such as the interfollicular area and the T–B-cell border.[6] Here the second phase of their differentiation occurs. In this phase the T–B-cell interaction is symbiotic, providing both cell types with signals required to support the differentiation that enables both cell types to participate in the GC

reaction. The activated B cells provide further antigenic stimulation and co-stimulatory and co-inhibitory signals (including; CD80, CD86, ICOSL, OX40L, PD-L1, PD-L2), enabling the pre-Tfh cells to complete differentiation and move into the GC. In turn, the pre-Tfh cell provides help to B cells by secreting cytokines [interleukin-21 (IL-21) and IL-4] and expressing CD40L, that can prompt B-cell follicular entry. The third phase occurs in the GC where Tfh cells engage with GC B cells providing them with help to ensure their proliferation, and subsequent differentiation into plasma and memory B cells.[3,4,7] The antigenic and co-stimulatory signals that direct each of the three phases of Tfh cell differentiation will be outlined in this review.

The role of antigen presentation in Tfh cell differentiation

Strength and duration of T-cell receptor signal

Although high-affinity $CD4^+$ T-cell clones and T-cell receptors (TCR) with the strongest peptide–MHCII binding show skewed differentiation into Tfh cells, this is most likely due to increased clonal expansion in response to high-affinity antigens.[8,9] It has been suggested that although antigen affinity does not correlate with partitioning to Tfh cell fate, different peptide epitopes may dictate T helper type 1 (Th1) versus Tfh cell differentiation.[10] Intriguingly, single cell transfer demonstrated that individual polyclonal T cells specific for a single peptide tend to produce progeny with a specific balance of Th1 and Tfh cells.[11] This divergence was due to enhanced Tfh differentiation with increased aggregate dwell time (the half-life for which a TCR productively binds to its cognate pMHCII ligand).[11] Work using a synthetic system where TCR and peptide–MHC were replaced with hybridizing DNA strands showed that signalling is initiated when single bound TCR are converted into clusters of bound TCRs.[12] Longer dwell times and higher ligand densities synergize to promote TCR clustering. This increases the probability of TCR phosphorylation and ZAP-70 recruitment, resulting in appropriate downstream TCR signalling.

Unlike other T helper subsets, Tfh cells require continuous antigenic stimulation for their maintenance. Experimental strategies to prolong antigen presentation by DC leads to increased numbers of Tfh cells in mice.[13,14] In humans, use of high antigen dose vaccines also results in increased antibody levels and circulating Tfh-like cells.[15–17] In autoimmune settings, chronic antigen exposure correlates with increased numbers of Tfh cells.[18–21] Likewise, increased proximal TCR signalling (e.g. in PTPN22-deficient mice) results in increased proliferation and accumulation of Tfh cells.[22] In our own studies we find that continuous antigenic stimulation is necessary for human Tfh differentiation *in vitro* (Webb and Linterman unpublished observation), demonstrating the dependence of Tfh cells on continuous antigen stimulation.

Presentation of antigen by DC

Antigen is presented to naive $CD4^+$ T cells by DC. This initial T–DC interaction results in the induction of Bcl6, the transcriptional repressor required for Tfh formation.[23–25] DCs are essential for Tfh induction, with B cells becoming the major antigen-presenting cell type for Tfh cells in the second and third phases of their differentiation.[26,27] In comparison to signals that regulate the B–Tfh cell interaction relatively little is known about the signals required to generate Tfh cells during the first DC–T-cell interaction. However, in conditions of high antigen dose such as viral infection, DC are dispensable for the generation of Tfh cells, suggesting that they are only essential when the amounts of antigen are limiting.[27,28] The mode of antigen presentation, the co-receptors and the cytokines expressed by DC are key determinants of Tfh cell differentiation. Further rounds of antigenic stimulation in the second phase of Tfh cell differentiation, usually mediated by B cells, are required to stabilize Bcl6 expression and complete Tfh cell differentiation.[29]

Presentation of antigen by B cells

B cells play an essential role in supporting Tfh differentiation. Depletion of B cells or disruption of their ability to present antigen results in a substantial reduction in Tfh cell numbers.[23,29–31] This is not due to a unique B-cell signal because the defect can be overcome by boosting with antigen and/or prolonged antigen presentation by DC.[32] Recent work has shown that B cells produce Ephrin B1 to repulse Tfh cells from the GC, thereby restricting their access to B cells and ensuring clonal competition.[33] In the absence of Ephrin B1, the Tfh cell production of IL-21 is reduced and fewer plasma cells are generated. The TCR signalling triggered in pre-Tfh cells by B cells results in prolonged calcium signalling, inducing the cytokines IL-4 and IL-21.[34] Qualitatively, this is a different response to that elicited during antigen presentation by DC, probably due to the increased size and duration of the synapses formed between pre-Tfh and B cells. Calcium signalling downstream of the TCR is essential for Tfh cell development; T cells that have a reduced ability to release Ca^{2+} (due to deficiency in both Stim1 and Stim2) do not form Tfh cells.[35] Nuclear factor of activated T cells (NFAT) transcription factors are activated by TCR-induced Ca^{2+} signalling and pre-Tfh cells have enhanced NFAT nuclear localization.[36] Genetic ablation of both NFAT1 and NFAT2 results in a T-cell intrinsic defect in Tfh cell generation.[37] This is not due to a general defect in T-cell activation as Th1 cell generation was

elevated in the absence of NFAT1 and NFAT2. In humans, nearly half of genes differentially expressed in Tfh cells possess NFAT binding sites near their transcriptional start sites (including *ICOS*, *CXCR5* and *SLAMF1*), suggesting that NFAT is a global regulator of Tfh cell differentiation, induced by antigen presentation during T–B-cell interactions.[37]

The importance of co-receptor signals in Tfh cell differentiation

Antigen presentation provides the antigen-presenting cells with an opportunity to further regulate Tfh cell differentiation through co-receptor interactions. Many co-receptors act as rheostats, tuning the magnitude of antigenic responses. Tfh cells express high levels of many co-receptors, a reflection of the sustained multi-signal pathways necessary for their generation and function.[3,4] In particular, CD28, cytotoxic T-lymphocyte antigen 4 (CTLA-4) and ICOS are essential for Tfh cell biology. They work sequentially along with OX40, PD-1 and signalling lymphocytic activation molecule (SLAM) family receptors (SFR) to regulate TCR signalling events, and initiate specific signalling pathways essential for Tfh differentiation.

CD28, CTLA-4 and ICOS receptor family

CD28, CTLA-4 and ICOS arose through a tandem duplication of an ancestral gene.[38] Despite their common evolutionary origin, they perform distinct roles in T-cell biology; CD28 and ICOS are positive regulators of T-cell activation, whereas CTLA-4 negatively regulates T-cell expansion.[39] CD28 and ICOS share 39% of their amino acid identity, and have overlapping capacity to activate the phosphoinositide 3 kinase (PI3K) signalling pathway; however, they play distinct roles in Tfh cell differentiation.[40] CD28 is highly expressed on naive and resting cells, whereas expression of ICOS and CTLA-4 depends upon T-cell activation.[39] The distinct patterns of their expression partly define their individual roles, but there are also differences in the signalling motifs in their cytoplasmic tails.[41] CD28 is the main inducer of IL-2, critical for the early growth phase of recently activated T cells, and is also an inhibitor of late Tfh cell differentiation.[42,43] In contrast, ICOS is a poor inducer of IL-2.[44,45] Neither CD28 nor ICOS co-stimulation alone are sufficient but rather but work sequentially to drive Tfh cell development.[46,47]

The strength of CD28 signalling *in vivo* translates directly into the level of ICOS expression on the T cells.[46] CD28 co-stimulation also induces expression of PD-1, OX40 and CXCR5.[46] Expression of CXCR5 allows pre-Tfh cells to respond to CXCL13 and migrate into B-cell follicles.[48] When CD28 signalling is blocked at the time of T-cell priming, T-cell activation is suppressed and this prevents Tfh cell differentiation *in vivo*.[49] CD28 co-stimulation depends upon the RLTPR, a scaffold protein that links CD28 to the CARD11/CARMA1 cytosolic adapter and to the nuclear factor-κB (NF-κB) pathway.[50,51] Mice and humans deficient in RLTPR have very few Tfh cells and this is associated with defects in activation-induced expression of CD40L, ICOS and RelA phosphorylation (indicative of impaired NF-κB signalling).[50,51] Once T cells have acquired CXCR5 expression and migrated to B-cell follicles the role of CD28 is less clear. Tfh cell differentiation can occur when CD28 co-stimulation is inhibited after priming *in vivo* by administration of CTLA-4–immunoglobulin, a treatment that would also prevent CTLA-4 signalling.[46,52] However, deletion of CD28 expression after T-cell priming results in fewer Tfh cells and increased Tfh cell death following influenza virus infection suggesting that CD28 is required up until the third phase of Tfh cell differentiation.[53] Importantly, ICOS expression in *Cd28*$^{-/-}$ T cells does not rescue the decrease in Tfh cell numbers, suggesting that CD28 stimulation provides unique signals essential for Tfh cells.[53]

CTLA-4 is expressed at high levels on Tfh cells where it imparts a negative signal to restrain their numbers.[46,52] CTLA-4-deficient mice show a skewing towards Tfh differentiation, with induction of IL-21 production and spontaneous GC formation.[46,54] CTLA-4 exerts its suppressive effects through cell extrinsic and cell intrinsic mechanisms. It reduces the expression of CD80 and CD86 (co-stimulatory ligands for CD28) on antigen-presenting cells through transendocytosis and its ligation inhibits T-cell proliferation and IL-2 transcription.[55] The cytoplasmic tail of CTLA-4 interacts with the Src homology domain-containing tyrosine phosphatases SHP1, SHP2 and PPS2, which dephosphorylate key TCR signalling kinases (Fyn, Lck and ZAP-70) and members of the Ras pathway.[56] By preventing CD28 co-stimulation, limiting ICOS expression and directly suppressing TCR signalling, CTLA-4 acts as a brake for Tfh cell differentiation.[52,56,57]

ICOS is critical for the GC response and is highly expressed on Tfh cells.[58] In the absence of ICOS, reduced Tfh cell numbers are seen in both mice and humans.[59–63] ICOS signalling is required for the maintenance of many characteristics of Tfh cell identity, including Bcl6 expression and IL-21 production. Impaired negative regulation of ICOS by the E3 ubiquitin ligase Roquin leads to abberant accumulation of Tfh cells.[64–66] A limited GC response can be mounted in *Icos*$^{-/-}$ mice; however, once GC are established the ICOS–ICOSL interaction becomes critical and *Icos*$^{-/-}$ mice have no GC following secondary challenge.[60–62,67] ICOS-deficient T cells are unable to enter B-cell follicles and this cannot be overcome by transgenic expression of CXCR5, suggesting that ICOS signalling imparts some CXCR5-independent motility to Tfh cells.[68]

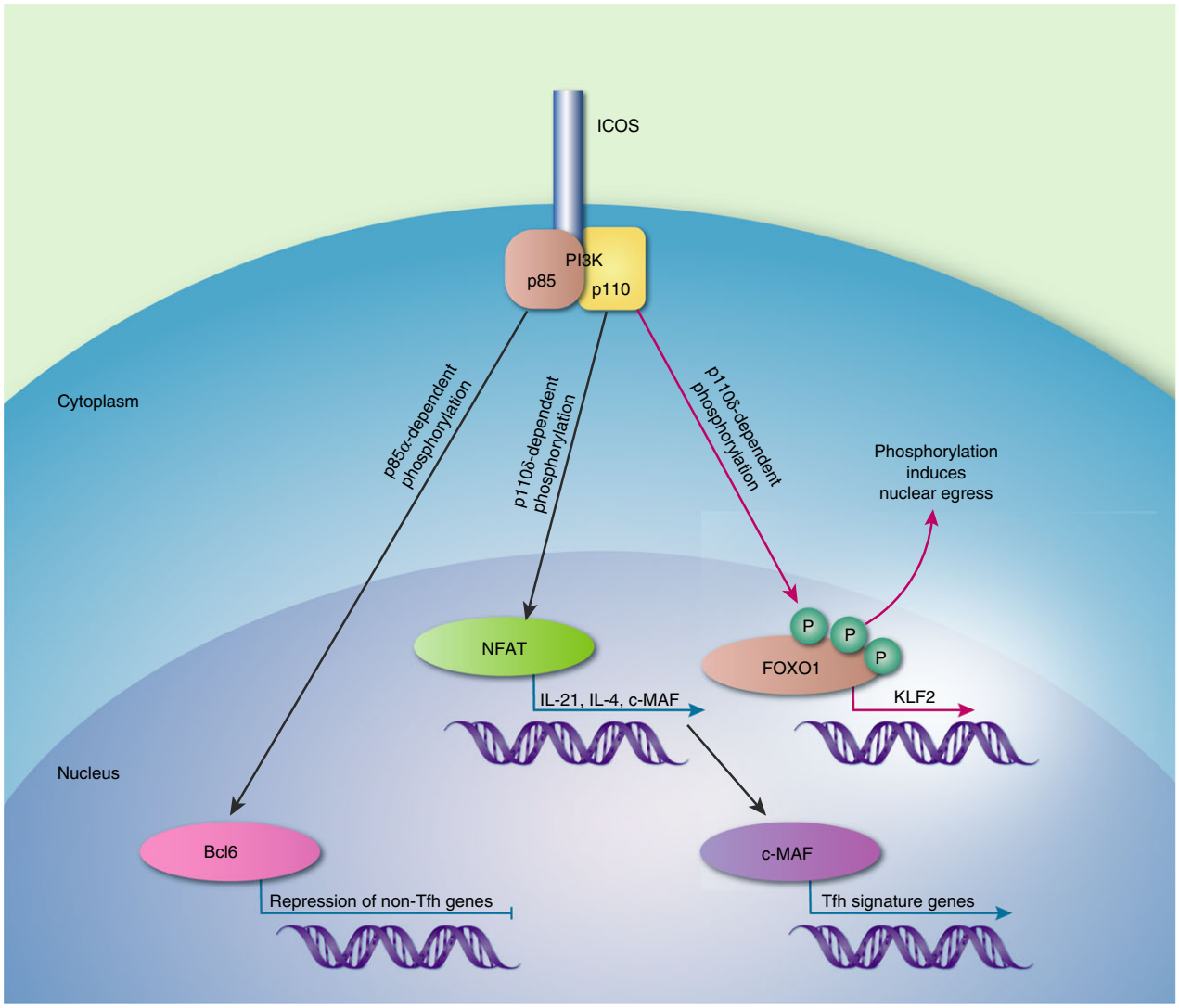

Figure 2. The roles of phosphatidlyinositol 3-kinase (PI3K) subunits in inducible T-cell co-stimulator (ICOS) -mediated T follicular helper (Tfh) cell differentiation. The regulatory p85α subunit forms a complex with osteopontin (OPN). This complex migrates to the nucleus to protect B-cell lymphoma 6 (Bcl6) from degradation. Signals through the catalytic p110δ subunit are responsible for nuclear factor of activated T cell (NFAT) activation allowing transcription of interleukin-21 (IL-21), IL-4 and c-MAF. The transcription factor c-MAF drives the expression of Tfh cell genes. PI3K also induces phosphorylation of FOXO1, promoting its nuclear egress and so preventing it from activating the transcription factor KLF2, which inhibits Tfh cell differentiation.

Within the GC, ICOS–ICOSL interactions promote extensive cell surface engagement of Tfh cells with B cells, resulting in Tfh cell calcium spikes and B-cell acquisition of CD40 signals, a feed-forward loop that promotes further ICOS expression and provision of help to B cells.[69]

PI3K signalling is crucial for ICOS function in Tfh cells.[70] A summary of the role of PI3K signalling following ICOS co-stimulation is depicted in Fig. 2. Mutations in the cytoplasmic tail of ICOS that abrogate recruitment of PI3K impair the generation of Tfh cells.[71] There is also a direct correlation between the magnitude of PI3K signalling and Tfh cell numbers.[72] PI3K signalling can be mediated through a number of different catalytic subunits. The p110δ catalytic subunit of PI3K transmits some of the signalling downstream of ICOS, principally by recruitment of PI3K to tyrosine within its cytoplasmic YFMF motif.[71,72] Like ICOS-deficient T cells, p110δ-deficient T cells show normal priming but are unable to enter primary follicles, and so numbers of Tfh cells are substantially reduced in GC.[72] Those that do form have defects in ICOS-mediated IL-21, IL-4 and cMaf mRNA expression.[72] The guanine nucleotide exchange factor Vav1 contributes to PI3K activation in T cells. *Vav1*$^{-/-}$ T cells are unable to provide B-cell help during the GC response and show defects in IL-4 and c-Maf mRNA expression.[73,74] However, ICOS-dependent up-regulation

of Bcl6 expression and development of $CXCR5^+$ Tfh-like cells is unaffected in p110δ-deficient mice.[71,72] Instead these events are dependent upon the p85α component of PI3K, which forms a complex with osteopontin and moves to the nucleus to protect Bcl6 from ubiquitination-dependent proteosomal degradation.[75] ICOS is superior to CD28 for regulating expression of the transcription factor Klf2.[76,77] This is probably because ICOS preferentially recruits the p110/p50α isoform of PI3K that is known to have an elevated lipid kinase activity and so a higher potential to promote phosphorylation of the transcription factor FOXO1.[78] This induces the nuclear exclusion of FOXO1, rendering it functionally inactive. This is critical because FOXO1 suppresses Tfh cell differentiation through negative regulation of Bcl6 and positive regulation of Klf2 expression.[76,77] ICOS also induces Akt (which also mediates phosphorylation of FOXO1) and the E3 ubiquitinase, ITCH, which degrades FOXO1.[79] This demonstrates that there is redundancy in the pathways downstream of ICOS that suppress FOXO1, to promote Tfh cell differentiation.

The expression of ICOS is tightly controlled. This is partly achieved through post-transcriptional mechanisms that regulate the stability of ICOS mRNA. RNA-binding proteins Roquin 1 and Roquin 2 bind to the 3′ untranslated region of ICOS mRNA, facilitating its degradation.[66,80] Mice with a single mutation in the Rc3h1 locus (*Sanroque*, which codes for a mutant form of Roquin 1 which is able to bind target mRNA but unable to direct mRNA degradation) and mice deficient in both Roquin 1 and Roquin 2 have increased levels of ICOS expression in all T cells, including naive T cells.[64,81] This results in increased numbers of Tfh cells and the generation of spontaneous GC. In addition, ICOS expression is regulated by the micro-RNA, MiR-146a.[82] Micro-RNAs target mRNAs for degradation and/or suppress their translation.[83] MiR-146a is highly expressed in Tfh cells and in its absence there is an accumulation of Tfh cells.[84] MiR-146a represses multiple canonical Tfh cell transcripts including *Icos*, *Slamf1*, *Cd84*, *Stat1*, *Cxcr4* and *Notch1*. Roquin 1 enhances dicer-mediated processing of MiR-146a, resulting in increased numbers of Tfh and GC B cells and increased expression of key Tfh mRNAs including ICOS. MiR-17-92 also regulates Tfh cell differentiation by restraining expression of genes important for the differentiation of other T-cell lineages.[85] It also promotes ICOS signalling by reducing the levels of the PI3K repressors PTEN and PHLPP2, thereby facilitating PI3K signalling.[85]

OX40

OX40 is expressed on activated T cells and disruption of OX40–OX40L interactions during the peak of the immune response perturbs Tfh cell differentiation.[86–88] Transgenic mice that overexpress OX40L on DC show increased numbers of Tfh cells, whereas OX40-deficient mice show impaired CD4 T-cell responses.[89–92] CD40-dependent maturation of DC results in the up-regulation of OX40L on T cells, although OX40–OX40L interactions are most instructive in the T–B-cell conjugates at later times of the immune response.[93,94] The role of OX40 in Tfh cell differentiation appears to be dependent upon the context of the immunization. Augmenting OX40 signalling during early stages of lymphocytic choriomeningitis virus infection can direct the CD4 T-cell response away from a Tfh cell fate.[95] OX40 signalling can also induce terminal differentiation of CD4 cells and enhance CD8 cell lytic capability in a tumour environment.[96,97] OX40 co-stimulation increases T-cell proliferation and CXCR5 expression during the later stages of activation, contributing to the aberrant Tfh cell response in systemic lupus erythematosus.[89,98–100] *In vitro* OX40 co-stimulation induces expression of Tfh cell-associated molecules and confers B-cell helper function.[100] However, this can be mimicked by simply increasing the level of TCR signalling, implying that OX40 merely amplifies the TCR signal. In agreement with this, OX40-deficient humans have normal Tfh differentiation and antibody responses.[101]

Like ICOS, OX40 is a strong activator of the PI3K, AKT and NF-κB signalling pathways.[71,94,102,103] It also enhances NFAT accumulation in CD4 T cells during antigenic stimulation.[104,105] OX40 is also a target of Roquin-1 and Roquin-2 through direct binding of their mRNA and 3′ untranslated region-dependent post-transcriptional repression.[106] Combined ablation of Roquin1 and Roquin2 induced the expression of OX40 and the activation of the alternative NF-κB pathway, resulting in elevated expression of Relb and IRF4.[106]

PD-1

T-cell activation results in PD-1 expression.[107,108] It is highly expressed by Tfh cells, memory T cells and exhausted CD8 T cells and its function may be cell-type-dependent.[30,109,110] Although the role of PD-1 in terminating T-cell responses and T-cell exhaustion have been studied extensively, its role in regulating Tfh cells is less well explored. PD-1 signalling is triggered by interaction with either PD-L1 (expressed on many cells, including activated B cells and Tfh cells) and PD-L2 (expressed on DC, macrophages and B-1 cells).[111,112]

Engagement of PD-1 results in the formation of microclusters of PD-1 with the TCR.[113,114] The tyrosine phosphatases, SHP1 and SHP2 are recruited to the intracellular tail of PD-1 where they decrease the phosphorylation status of CD3ζ chain immunoreceptor tyrosine-based activation motifs, attenuating ZAP-70 activation and inhibiting T-cell activation.[115–117] PD-1 engagement inhibits $CD4^+$ T-cell proliferation and

cytokine production.[118–120] These effects of PD-1 can be overcome by strong signalling through CD28 and/or IL-2R.[118,120–122] Recent work has highlighted the role that PD-1 plays in suppressing CD28 signalling.[123,124] CD28 is preferred over the TCR as a target for dephosphorylation by PD-1-recruited SHP2.[123] Consistent with this, the response to anti-PD-1 therapy requires CD28 signalling.[123] Hence it is the balance of the signals received from stimulatory and inhibitory receptors that determines the final outcome of T-cell fate.

Metabolic studies of CD8 T cells receiving PD-1 signals showed that they are unable to engage in glycolysis, glutaminolysis or metabolism of branched-chain amino acids and display an increased rate of fatty acid oxidation.[125] They also have substantial spare respiratory capacity, allowing production of energy under conditions of stress.[125] PD-1 ligation can also alter the metabolic programme of pre-activated $CD4^+$ T cells, reprogramming their metabolism from glycolysis to fatty acid oxidation thereby preventing effector cell development. The bioenergetic properties of PD-1 stimulated T cells display similarities to those of memory T cells, which sustain their survival due to catabolic metabolism of fatty acid oxidation. Whereas the effect of PD-1 stimulation in Tfh cells remains unknown, it is tempting to speculate that it prevents excessive proliferation and provides them with longevity. Indeed, comparison of Tfh and Th1 cells generated during acute viral infection showed that Tfh cells are less proliferative than Th1 cells.[36] This is accompanied by a reduction in glycolysis and an inability to maximally engage in aerobic glycolysis while maintaining IL-21 secretion. In addition, Bcl6 has been shown to repress the expression of genes involved in glycolysis.[126] Furthermore, single-cell RNA-Seq of T cells during malaria infection showed that at the bifurcation point where T cells differentiate into either Th1 or Tfh cells, Th1 cells cycled faster and expressed more genes associated with glycolysis than their Tfh counterparts.[127] The high levels of PD-1 expressed on Tfh cells may reduce glycolysis in these cells, resulting in maintenance of their phenotype with sustained cytokine production in the B-cell follicle. Mice deficient in both PD-L1 and PD-L2, or PD-1 show diminished numbers of long-lived plasma cells and higher levels of GC B-cell death following immunization.[128] This result is linked to increased numbers of Tfh cells and decreased IL-4 and IL-21 mRNA levels in these Tfh cells.[128] In addition, regulatory B cells express high levels of PD-L1, which attenuates T-cell activation and regulates Tfh cell differentiation [129].

SLAM

The role of SLAM in Tfh cell differentiation came to light with the realization that mutations in SLAM-associated protein (SAP) result in a lymphoproliferative disease. Patients with mutations in SAP have no natural killer T cells and impaired humoral immunity characterized by reduced Tfh cell differentiation.[130] SAP-deficient mice have a T-cell-intrinsic defect in humoral responses, characterized by poor GC formation, low antibody titres and a scarcity of memory B cells and long-lived plasma cells.[131,132] SAP is an adapter protein that can be recruited by SFRs. There are seven SFRs expressed on haematopoietic cells: 2B4, Ly9, CRACC, CD48, SLAM, CD84 and Ly108.[133,134] However, SFRs are able to signal through other SH2 domain-containing molecules (such as Fyn), particularly in the context of SAP deficiency.[135,136] Intravital imaging showed that SAP-deficient T cells are effectively activated by antigen-bearing DC but are unable to maintain stable conjugates with antigen-specific B cells, resulting in a poor GC response.[131] Tfh cells express high amounts of the SFRs CD84 and Ly108.[137,138] *Cd84*$^{-/-}$ mice show a reduction in GC formation and impaired humoral responses, most likely due to their inability to form stable B–T-cell conjugates.[138] Mice deficient in SLAM are defective in IL-4 production, suggesting that formation of T–B-cell conjugates enables Tfh cell cytokine production.[138] Yet the severe defect in GC formation seen in SAP-deficient mice has not been recapitulated in any single SLAM family receptor knockout mouse and deletion of all seven SFRs has no effect on the GC response.[139] Interestingly, Chen *et al.* showed that in the absence of SAP, SFR signalling is inhibitory in Tfh cells and suppresses humoral immunity.[139] Genetic deletion of Ly108 reverses the phenotype of SAP-deficient mice.[137] Ly108 can associate with both SAP and SHP-1 and both molecules compete for the same immunoreceptor tyrosine-switch motif suggesting that Ly108 can act as a rheostat for T–B-cell interactions. In Tfh cells, antibody-mediated cross-linking of SFRs induces the phosphorylation of tyrosine residues on SHP1 and the biochemical suppression of SHP1 can alleviate SFR-mediated inhibition in SAP-deficient T cells.[139] Hence, it appears that SAP works by preventing the coupling of SFRs to inhibitory signalling pathways, but that this activity is not required for T–B-cell conjugates that form in the GC.

Conclusions

Co-receptors provide critical and unique signals to drive effective Tfh cell differentiation. They can act as rheostats for TCR signalling, tempering or elevating the intracellular signals transmitted following antigen engagement. They also provide signals to guide the migration of emerging Tfh cells and regulate expression of transcription factors, cytokines and co-receptors. The activation status and location of T cells within the environment of the secondary lymphoid organ determines which co-receptors are engaged and how. In the first phase of Tfh differentiation, DC provide the antigenic stimulus. This is

accompanied by CD28 co-stimulation, which drives Tfh differentiation through proliferation and expression of other co-receptors. This allows the emerging Tfh cell to respond to cytokines, migratory signals and further co-stimulatory and inhibitory signals. During the second and third phases of Tfh differentiation ICOS, OX40, CD40L and SFRs provide stimulatory signals that not only amplify antigenic signals but also provide unique signals that are critical for driving Tfh differentiation. The activation-induced expression of the inhibitory receptors CTLA-4 and PD-1 are critical during these stages of differentiation, enabling tight control of Tfh cell proliferation, critical for optimal immune responses. The evolution of B cells able to produce high-affinity antibodies depends upon competition among B-cell clones. This is partly achieved by limiting Tfh cell help. In conditions where there are excessive numbers of Tfh cells there is a lower selection pressure so low-affinity and self-reactive B-cell clones are not purged from the GC. Furthermore, in conditions where Tfh cell numbers are not restricted (e.g. when the PD-1 signalling pathway is blocked) mRNA expression of key cytokines is reduced and there are fewer resultant antibody-secreting cells and memory B cells. Restricting Tfh cell proliferation provides a limited number of cells able to provide high-quality help to B cells. Understanding the mechanisms used to govern this will underpin immunization strategies, selecting more stringently for the development of 'fit' Tfh cells and aiding development of treatments for diseases with aberrant Tfh cell function.

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Disclosure

The authors state that there are no competing interests.

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