**Supplementary Table 1: Effects of Gene-deficiency on Tfh cell formation**

| | Gene/Protein | Effect of ko/mutation on Tfh cells | Comment/potential or actual mechanism | References |
|---|---|---|---|---|
| **Cytokines** | IL-6 | • mild/moderate decrease<br>• no effect | • variable results probably reflects different immunisations and infections (eg acute vs chronic LCMV; influenza)<br>• combined deficiency of IL-6 and IL-21 reduced Tfh numbers *in vivo*, consistent with their ability to induce Bcl-6 and c-Maf | 1-6 |
| | IL-21, IL-21R | • mild decrease<br>• no effect | | 1,3-5,7-11 |
| | IL-27Ra | • decreased | • induces c-Maf, ICOS, IL-21<br>• promotes Tfh cell survival | 12,13 |
| | IL-2 | • increased | • induces Blimp-1 which suppresses Tfh commitment | 14,15 |
| | IL-10/IL-10R | • increased | • IL-10 suppresses Tfh formation directly by acting on naïve $CD4^+$ T cells but also indirectly by suppressing production of cytokines (IL-6, IL-23) by DC that may promote Tfh formation. Molecular mechanism of inhibitory action of IL-10 is unknown. | 16-18 |
| **Signalling** | *SH2D1A* (SAP) | • decreased/non-funcitonal | • requirement for SAP in Tfh cell can be overcome by providing excess Ag to circumvent the dependency on B cells as the primary APC<br>• however SAP-deficient Tfh cells are still unable to "help" cognate B cells due to an inability to form stable T-B conjugates<br>• mutations in *SH2D1A* results in impaired Ab production in humans due to a $CD4^+$ T cell intrinsic defect | 19-24 |
| | STAT1 | • transient decrease | • functions downstream of IL-6 receptors; greater defect in Tfh formation when naïve CD4+ T cells lack both STAT1 and STAT3 | 6 |
| | STAT3 | • decreased | • role of STAT3 probably accounts for redundancy in the effects of IL-6 or IL-21 deficiency on Tfh formation<br>• mutations in *STAT3* results in a deficiency in production of Ag-specific Ab, memory B cells and circulating $CD4^+CXCR5^+$ T cells in humans; STAT3 probably acts downstream of IL-6, IL-21 as well as IL-12 in human CD4+ T cells to induce Tfh cells | 1,25,26 |
| | STAT4 | • transient decrease | • IL-12/STAT4 signalling induced T-bet which suppresses Bcl-6 function | 27 |
| | STAT5 | • increased | • directly binds Bcl6 to inhibit expression<br>• activated by IL-2, which induces Blimp-1 to suppress Tfh commitment | 14 |
| | NIK (NFkB inducible kinase) | • decreased | • NIK-dependent signaling downstream of BAFF-R on B cells is required for constitutive expression of ICOS-L on these cells, which is required for Tfh formation | 28 |
| | Roquin | • increased | • mutation in *san roqin* gene causes severe lupus-like disease due to aberrant Tfh accumulation and subsequent formation of GCs and autoAb production | 29 |
| **Transcription factors** | Bcl-6 | • absent | • required for Tfh formation<br>• inhibits expression/function of T-bet, Gata3, RORγt and generaton of Th1/2/17 cells<br>• suppresses expression of clusters of miRNAs that inhibit Tfh formation<br>• considered the "master regulator" of Tfh cells, but likely to require actions of other transcription factors to induce Tfh cells | 4,30-32 |

| | | | | |
|---|---|---|---|---|
| | c-Maf | • decreased | • induces expression of IL-21, ICOS<br>• co-operates with Bcl-6 to induce/enhance expression of some key features of Tfh cells | 13,33-35 |
| | BATF | • absent | • functions by inducing Bcl-6 and c-Maf<br>• additional BATF-targets also likely to be involved in Tfh formation | 36,37 |
| | IRF4 | • decreased/absent | • co-operates with STAT3 to mediate IL-21-induce gene expression in $CD4^+$ T cells, however its exact role in Tfh cells remains unknown | 38 |
| | *PRDM1* (Blimp1) | • increased | • Blimp-1 restricts Tfh formation by antagonising Bcl-6 function | 30 |
| | *Tbx21* (T-bet) | • increased | • T-bet suppresses Bcl-6 function | 27,39 |
| **Surface receptors** | CD40/CD40L | • decreased/absent | • mutations in *CD40L* or *ICOS* result in impaired Ab production, memory B cell formation and reduced circulating $CD4^+CXCR5^+$ T cells in humans<br>• due to a CD4+ T cell intrinsic defect in the ability to form GC<br>• ICOS signalling can also induce c-Maf and IL-21 (although it is unknown whether ICOS directly induces IL-21 or indirectly by inducing c-Maf) | 40-42 |
| | ICOS/ICOS-L | • decreased/absent | | |
| | CD28 | • decreased/absent | • Tfh deficiency in CD28 ko phenocopied by CD86, but not CD80, deficiency; CD86 dominant over CD80 for Tfh formation. CD80 may have role in Tfh formation, but it is modest | 41,43,44 |
| | CD80 | • minimal/no effect | | |
| | CD86 | • decreased/absent | | |
| | CD84 | • decreased/no effect | • Tfh cells were reduced in $Cd84^{-/-}$ mice in response to immunization with protein Ag, but was less severe than SAP deficiency; however there was no defect in Tfh formation following viral infection. Thus additional SLAM receptors are also involved in SAP-dependent development of Tfh cells | 21,45 |
| | Ly108 | • no effect of single gene knockout | • although Ly108 deficiency had no effect on Tfh formation, the absence of both Ly108 and SAP restored the Tfh defect observed in SAP-deficient mice. This revealed the ability of Ly108 to deliver inhibitory signals via SHP-1 which countered the positive signals delivered through other receptors (including SLAM family/SAP-associating receptors) on Tfh formation | 45 |
| | OX40/OX40L | • decreased/<br>no effect | • OX40 signalling is required for induction of CXCR5 expression on activated $CD4^+$ T cells and their subsequent relocation from the T zone to the B-cell follicle<br>• OX40 expression on $CD4^+$ T cells depends on CD28 signalling, while OX40L expression on DCs requires engagement of CD40; this places OX40/OX40L interactions downstream of CD28/B7 and CD40L/CD40 interactions between CD4+ T cells and APCs | 41,46-49 |
| | TACI | • increased | • BAFF and APRIL-mediated signals through TACI function to intrinsically suppress ICOS-L expression on B cells | 50 |
| | PD-1 | • increased | • PD-1 negatively regulates Tfh formation; PD-L1 on B cells is the predominant ligand providing the inhibitory signal to constrain Tfh development via PD-1<br>• depending on the experimental model, the increase in Tfh cells in the absence of PD-1 signalling can result in either enhanced or diminished GC and Ab responses<br>• PD-1 may preferentially suppress formation of follicular Treg, rather than Tfh, cells | 51-55 |

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