of Parkinson disease–linked α-synuclein are shuttled within neurons for ultimate degradation and are processed before their release or following their uptake in the context of LRRK2-Rab interactions, as well as how these interactions promote neurodegeneration, promises to inform the identification of targets for future therapy of Parkinson disease and related disorders.

Another interesting point is the potential role of the Nod2-LRRK2-Rab2a axis in cells of the immune system. Various cells of the immune system, including both myeloid cells and lymphoid cells, have relatively high LRRK2 expression[17]. In both CD and Parkinson disease (as well as leprosy), the potential effect of aberrant inflammation in the initiation and progression of disease is of enormous interest. Further investigation into how cargo sorting and maturation of secretory granules in cells of the immune system are affected by mutations at two loci, *NOD2* and *LRRK2*, could be a novel angle of research into the pathogenesis of CD and Parkinson disease. Thus, the findings of Zhang *et al.* identify a new paradigm in host-microbiota homeostasis in which two CD-associated proteins, Nod2 and LRRK2, participate in a common axis important for the proper secretion of lysozyme by Paneth cells to ensure protection of the intestinal barrier.

COMPETING FINANCIAL INTERESTS
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TCF-1 and LEF-1 help launch the T_{FH} program

Masato Kubo

Follicular helper T cells (T_{FH} cells) differentiate from naive T cells, but the picture of this differentiation process remains incomplete. Two studies now identify the related transcriptional regulators TCF-1 and LEF-1 as important early participants in this process.

Follicular helper T cells (T_{FH} cells) are a specialized subset of T cells that provide signals necessary for antigen-specific B cells to generate the germinal center (GC). This structure is required for class-switch recombination, somatic hypermutation and the selection of B cells that produce high-affinity antibodies, as well as for the generation of long-lived antibody-secreting plasma cells and memory B cells[1]. A distinguishing marker of T_{FH} cells is the chemokine receptor CXCR5, which is required for their entry into B cell follicles. T_{FH} cells access the follicle by upregulating CXCR5 and by downregulating CCR7 and P-selectin glycoprotein ligand 1 (CD162). The T_{FH} cells with the highest expression of CXCR5 and another marker, PD-1, seem to 'preferentially' accumulate in GCs by sensing the CXCR5 ligand CXCL13 (refs. 2,3) (**Fig. 1**). T_{FH} cells also express several costimulatory molecules, including CD40L, ICOS, OX40 and members of the SLAM family, all of which have important roles in T cell–dependent B cell responses driven by cognate T cell–B cell interactions[2,3]. T_{FH} cells are also the source of interleukin 21 (IL-21) and IL-4, cytokines that are necessary for class-switch recombination. In this issue of *Nature Immunology*, Lifan Xu *et al.*[4] and Youn Soo Choi *et al.*[5] identify the transcriptional regulators LEF1-1 (encoded by *Lef1*) and TCF-1 (encoded by *Tcf7*) as important participants early in the T_{FH} developmental process (**Fig. 1**).

The lineage commitment of T_{FH} cells begins with the initial priming of naive $CD4^+$ T cells by dendritic cells or by other myeloid cell–derived antigen-presenting cells in the T cell zone (**Fig. 1**). After a few rounds of cell division, these T cells express Bcl-6, a transcriptional repressor that belongs to the BTB-POZ family, whose function is critical for T_{FH} differentiation, as indicated by the complete absence of T_{FH} cells among *Bcl6*$^{-/-}$ $CD4^+$ T cells[6–8]. Moreover, ectopic *Bcl6* expression in $CD4^+$ T cells reconstitutes the generation T_{FH} cells. Bcl-6 expression in B cells is also critical for T_{FH} development, because T_{FH} cells are again abrogated in mice with conditional deficiency of Bcl-6 in B cells[8]. Those observations indicate that the transcriptional program of Bcl-6 seems to be controlled via priming by antigen-presenting cells.

It is important both for basic knowledge and for vaccine development to understand how the T_{FH} program, including regulation of *Bcl6* expression, is controlled. Cytokine pathways, such as IL-6–STAT1, IL-12–STAT4 and IL-21–STAT3, have been linked to the induction of T_{FH} cells and *Bcl6* expression[2]. Batf and Ascl2 are critical transcription factors that support T_{FH} differentiation by controlling the expression of *Bcl6* and *Cxcr5*, respectively, which suggests that Bcl-6 and CXCR5 are regulated by independent molecular mechanisms. Despite such advances, the gap between the regulation of *Bcl6* expression and the T_{FH} program is still puzzling, because there are no obvious mechanisms by which Bcl-6 controls transcriptional programs for T_{FH} differentiation, and this makes it likely that other unknown factors are involved.

Choi *et al.*[5] and Xu *et al.*[4] identify two additional participants in the T_{FH} program: LEF1-1 and TCF-1. These are members of the TCF-LEF subfamily and belong to a family of high-mobility-group proteins that work as downstream repressors of the canonical Wnt signaling pathway. TCF-1 controls mainly the proliferation and population expansion of thymocytes; thus, lack of TCF-1 causes a substantial reduction in thymic cellularity and a partial block in thymocyte differentiation at the transition from the $CD8^+$ immature single-positive stage to the $CD4^+CD8^+$ double-positive stage. Although LEF1-1-deficient mice have normal T cell development in the thymus, deficiency in both LEF1-1 and TCF-1 leads to a complete block in differentiation[9,10]. TCF-1 and LEF-1 are also involved in the function and formation of memory $CD8^+$ T cells[11]. In $CD4^+$ T cells, TCF-1 is reported to be a

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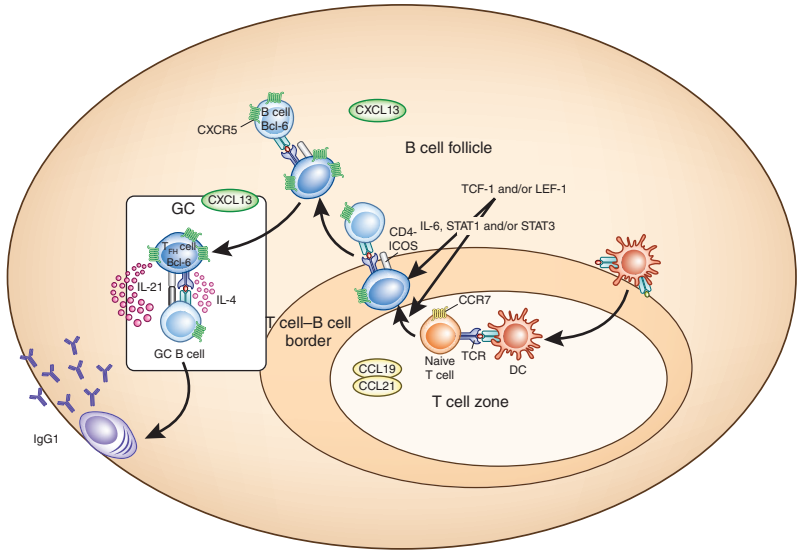

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Figure 1 LEF-1 and TCF-1 control early steps in the T_{FH} developmental process. LEF-1 and TCF-1 are positive regulators that control the expression of *Bcl6, Il6ra, Il6st* and *Icos*, which encode products known to be required for T_{FH} differentiation. IgG1, immunoglobulin G1; CCL19 and CCL21, chemokines; TCR, T cell antigen receptor; DC, dendritic cell.

positive regulator of the expression of genes encoding the transcription factor GATA-3 and IL-17A and a negative regulator of expression of the gene encoding interferon-γ[12].

Choi *et al.* perform an unbiased transcriptome analysis of early T_{FH} cells and the T_H1 subset of helper T cells to identify transcription factors that control the T_{FH} program[5]. They identify *Lef1* because it satisfies two further criteria: its 'preferential' expression in early T_{FH} cells *in vivo*, and the large effect on differentiation into fully committed GC T_{FH} cells and the generation of GC B cells when it is ablated. These authors further demonstrate high expression of *Tcf7* by early T_{FH} cells and that synergistic regulation by LEF-1 and TCF-1 is essential for the full T_{FH} cell program. Studies using a green fluorescent protein (GFP) reporter system (TCF-1–GFP) further support the proposal of the functional importance of TCF-1 in T_{FH} cells. The expression of TCF-1–GFP is highest in naive T cells but is lower in antigen-primed T cells and effector T cells. After infection with lymphocytic choriomeningitis virus, TCF-1–GFP expression is greatly diminished in T_H1 cells, while high expression is maintained in T_{FH} cells. Mature T cells with conditional deletion of *Lef1* and *Tcf7*, which lack both Lef-1 and TCF-1, generate considerably fewer fully committed GC T_{FH} cells than do their wild-type counterparts, which suggests that coordination of LEF-1 and TCF-1 is required for T_{FH} differentiation through the regulation of circuits upstream of *Bcl6*. The binding of LEF-1 and TCF-1 to several different genes consequently results in the promotion of *Bcl6* expression, sustained expression of the cytokine receptor subunits IL-6Rα and gp130 (IL-6st), and enhanced expression of ICOS to accomplish T_{FH} differentiation.

Xu *et al.* also find higher expression of TCF-1 in early committed T_{FH} cells after infection with lymphocytic choriomeningitis virus, in contrast to its decreased expression in T_H1 cells[4]. They also show diminished development of fully committed T_{FH} cells resulting from TCF-1 deficiency in two independent conditional deletion systems (T cell–specific deletion (*Cd4*-Cre) and tamoxifen-induced deletion (ERT2-Cre)), in system of chimeras reconstituted with a mixture of *Tcf7*$^{-/-}$ bone marrow and wild-type bone marrow , and in a system of short hairpin RNA–mediated knockdown. However, unlike Choi *et al.*[5], they find that TCF-1 needs no cooperation with LEF-1 in the T_{FH} program. Moreover, T_{FH} cells lacking TCF-1 show lower expression than TCF-1-sufficient cells of genes encoding several T_{FH} markers (including *Bcl6*, *Icos*, *Ascl2*, *Cxcr5*, *Il6ra*, *Il6st*, *Il21* and *Il4*) and instead show increased expression of non-T_{FH} cell signature genes (*Tbx21, Gzmb, Gata3*, *Rorc* and *Foxp3*); this suggests that TCF-1 is a cell-fate regulator that functions by suppressing the differentiation programs of non-T_{FH} effector cells.

Xu *et al.* further demonstrate that expression of TCF-1 is critical for the axis of Bcl-6 and the transcription factor Blimp-1 in T_H1-versus-T_{FH} cell-fate determination[4]. Studies of an *in vitro* 293T human embryonic kidney cell overexpression system indicate that co-expression of the p33 isoform of TCF-1, Bcl-6 and TLE3 (the dominant member of the TLE corepressor complex) in CD4$^+$ T cells enhances formation of the p33–Bcl-6 complex. These *in vitro* data indicate that TCF-1 binds both directly and indirectly to the *Bcl6* promoter to upregulate its expression, while indirect binding of the p33–Bcl-6 complex to 5′ regulatory regions of the gene encoding Blimp-1 represses Blimp-1 expression. Given these results, Xu *et al.* propose that TCF-1 is an upstream regulator of the Bcl-6–Blimp-1 axis required for T_{FH} differentiation[4].

These two studies raise some interesting questions about how the activity of TCF-1 and/or LEF-1 is able to influence the differentiation program of T_{FH} cells and other helper T cell subsets. Both studies agree on the point that TCF-1 is a positive regulator of Bcl-6 and a negative regulator of Blimp-1, but there is clear discrepancy in the proposed activity of LEF-1. Choi *et al.* suggest that LEF-1 is needed in a synergistic way with TCF-1 to regulate commitment to the T_{FH} lineage[5], while Xu *et al.* raise the conflicting possibility that LEF-1 may not necessarily lead to full commitment[4]. TCF-1 directly binds to the *Bcl6* promoter to control its expression regardless of LEF-1's binding, whereas TCF-1 can repress *Lef1* expression. TCF-1 is needed to inhibit Blimp-1 activity to stop differentiation into other helper T cell subsets. In the same context, TCF-1 is known to be positive regulator for the functions of T_H2 cells and T_H17 cells[11], which indicates that TCF-1 must have a complicated role to be able to provide positive differentiation signals for both T_{FH} cell subsets and non-T_{FH} cell subsets. Indeed, a published study has indicated that T_{FH} cells can differentiate from T_H2 cells able to express several T_{FH} cell signature genes[13]. Since both T_H2 cells and T_{FH} cells need TCF-1 activity for their differentiation, TCF-1 may provide the mechanism by which T_H2 cells have the potential to differentiate into T_{FH} cells. Choi *et al.*[5] and Xu *et al.*[4] propose several interesting molecular mechanisms downstream of TCF-1 and LEF-1, but it remains unclear whether the T_{FH} program is regulated by a single mainstream pathway or by the coordination of multiple pathways. Further research should focus on determining how molecules downstream of TCF-1 and LEF-1 control the T_{FH}-differentiation program.

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