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RESEARCH HIGHLIGHT

Critical role of PD-1/PD-L1 pathway in generation and function of follicular regulatory T cells

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The balance between follicular helper T cells (T_{FH} cells) and follicular regulatory T (Treg) cells (T_{FR} cells) is one of the key factors for the development of humoral immune responses. How the balance is regulated, however, is largely unknown. In a recent issue of *Nature Immunology*, Sage *et al.* revealed a critical role of the programmed death-1 (PD-1)/PD-L1 pathway in T_{FR} cell generation and function,[1] which provides a novel insight into the mechanisms how T_{FH} and T_{FR} cells regulate antibody production.

Germinal centers (GCs) are sites within lymph nodules, where B lymphocytes rapidly proliferate and differentiate into plasma cells and memory B cells during humoral immune responses. These events are precisely regulated by interactions between cognate B cells, T_{FH} cells, T_{FR} cells and follicular dendritic cells.[2–5] T_{FH} cells are a specialized $CD4^+$ helper subset, which provides help to B cells. T_{FR} cells are a newly identified $CD4^+$ Treg subset in GCs, which downregulates the GC response.[6,7] T_{FH} and T_{FR} cells are defined by expression of the CXC-chemokine receptor 5 (CXCR5) which directs them to B-cell follicles in GCs *via* gradients of the CXC-chemokine ligand 13 (CXCL13).[1,2,8] T_{FH} and T_{FR} cells are present not only in lymph nodes but also in peripheral blood. PD-1 is a well-known inhibitory receptor for humoral immune responses[9] and can be expressed by T cells, B cells, macrophages and dendritic cells.[10] T_{FR} cells express more PD-1 than T_{FH} cells.[1] B cells and dendritic cells express the PD-1 ligands, PD-L1 and PD-L2.[10]

To uncover how the balance between T_{FH} and T_{FR} cells controls humoral immunity, Sage *et al.* attempted to clarify the individual role of PD-1 expressed on T_{FH} and T_{FR} cells. Table 1 summarizes the major cells, molecules, antigens and knockout/transgenic mice and Figure 1 outlines the three main approaches and the findings of this work.

In the first approach, the authors determined the frequency and number of T_{FH} cells ($CD4^+CXCR5^+Foxp3^-ICOS^+CD19^-$) and T_{FR} cells ($CD4^+CXCR5^+Foxp3^+ICOS^+CD19^-$) in 4 strains of C57BL/6 mice: wild-type (WT), PD-1-deficient ($Pdcd1^{-/-}$), PD-L1-deficient ($Cd274^{-/-}$) and PD-L2-deficient ($Pdcd1lg2^{-/-}$), these mice being immunized with MOG. Deficiency of PD-1 or PD-L1, but not PD-L2, resulted in more T_{FR} cells than T_{FH} cells in lymph nodes and blood. Compared to WT T_{FR} cells, PD-1-deficient T_{FR} cells displayed a potent capacity to inhibit both the proliferation of responder T and B cells and the T_{FH} cell-mediated IgG production *in vitro*. The expressions of activation markers (CD25, CD69 and Ki67) and transcription factors (Bcl-6, Blimp-1, RORγt and IRF4) by T_{FH} and T_{FR} cells were also compared between $Pdcd1^{-/-}$ and WT mice, as shown in Figure 1. The data indicate that the PD-1/PD-L1 pathway controls not only the generation and function of T_{FR} cells but also the ratio of T_{FH} to T_{FR} cells. In addition, $Cd28^{-/-}$ and $Icos^{-/-}$ mice showed considerable deficiency of T_{FH} and T_{FR} cells, suggesting that CD28 and ICOS provide essential costimulatory signals for T_{FH} and T_{FR} cell development. Like WT T_{FR} cells, PD-1-deficient T_{FR} cells can home to GCs.

In the second approach, the authors investigated the role of PD-1/PD-L1 pathway in the differentiation of T_{FR} cells from $CD4^+Foxp3^+CXCR5^-$ Treg cells. They used 2D2 and $Pdcd1^{-/-}$2D2 mice that obtained transgenic expressions of a MOG-specific TCR and Foxp3-green fluorescent protein (GFP). The MOG-specific GFP^+ Treg cells purified from these mice were transferred into WT mice and the recipients were further immunized with MOG. In the mice receiving $PD\text{-}1^-$ Treg cells, the GFP^+ T_{FR} cells ($CD4^+Foxp3^+CXCR5^+$) in draining lymph nodes were found at higher frequency and higher absolute number than in the mice received $PD\text{-}1^+$ Treg cells. These data confirm that the PD-1/PD-L1 pathway controls the differentiation of $CD4^+Foxp3^+CXCR5^-$ Treg cells into T_{FR} cells.

In the third approach, the authors evaluated whether T_{FR} cells in blood could suppress antibody production *in vivo*. WT, $Pdcd1^{-/-}$ and $Cxcr5^{-/-}$ mice were immunized with NP-OVA. WT T_{FH} cells ($CD4^+ICOS^+CXCR5^+GITR^-CD19^-$), WT and PD-1-deficient T_{FR} cells ($CD4^+ICOS^+CXCR5^+GITR^+CD19^-$) and CXCR5-deficient Treg cells ($CD25^+CD62L^+$) were sorted from blood 8 days

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Table 1 Cell types, molecules, antigens and mice

Name		*Feature*
Cell	T_{FH} cell	Follicular helper T cell, $CD4^+CXCR5^+Foxp3^-ICOS^+Bcl\text{-}6^+CD19^-$ and $PD\text{-}1^+GITR^-$. T_{FH} cells are essential for GC formation and for B cell activation/differentiation into plasma cells
	T_{FR} cell	Follicular regulatory T cell, $CD4^+CXCR5^+Foxp3^+ICOS^+Bcl\text{-}6^+CD19^-$ and $PD\text{-}1^+GITR^+$. T_{FR} cells can inhibit T_{FH} cell function and naive T- and B-cell activation
	$CD4^+$ Treg cell	Treg cell, $CD4^+CXCR5^-Foxp3^+$ and $GITR^+$
Molecule	PD-1	PD-1 (CD279), also called PDCD-1. Well-known inhibitory receptor and expressed by T cells, B cells, macrophages and dendritic cells
	PD-L1	Ligand 1 of PD-1 (CD274), also called B7 homolog-1 (B7-H1)
	PD-L2	Ligand 2 of PD-1 (CD273), also called B7-DC
		PD-L1 and PD-L2 are type I transmembrane proteins related to the CD28 ligands, but do not bind CD28 or CTLA-4 (CD152). B cells and dendritic cells in GCs express both PD-L1 and PD-L2
	CXCR5	CXC-chemokine receptor 5 (CD185), a marker of T_{FH} and T_{FR} cells in a follicular program
	CXCL13	CXC-chemokine ligand 13, also called BLC. CXCL13 can direct the migration and relocation of $CXCR5^+$ B cells, T_{FH} cells and T_{FR} cells into follicles
	GITR	Glucocorticoid-induced tumor-necrosis factor receptor (CD357, TNFRSF-18), a type 1 transmembrane protein of the TNF receptor superfamily. GITR is expressed on T_{FR} cells but not on T_{FH} cells, and is used as a marker to isolate T_{FR} cells for functional study
	CD28	Well-known T-cell costimulatory molecule. Its natural ligands are CD80 (B7-1) and CD86 (B7-2). CD28 provides essential costimulatory signals for T_{FH} and T_{FR} cells
	ICOS	Inducible T-cell costimulator (CD278). Its ligand is CD275 (B7H). ICOS provides essential costimulatory signals for T_{FH} and T_{FR} cells
Antigen	MOG	Myelin oligodendrocyte glycoprotein peptide (amino acids 35–55) emulsified in complete Freund's adjuvant.
	NP-OVA	The hapten NP (4-hydroxy-3-nitrophenylacetyl) conjugated to ovalbumin.
Mouse	WT	WT C57BL/6
	Pdcd1$^{-/-}$	Deficient in PD-1
	Cd274$^{-/-}$	Deficient in PD-L1
	Pdcd1lg2$^{-/-}$	Deficient in PD-L2
	Cxcr5$^{-/-}$	Deficient in CXCR5, lacking a follicular program
	Cd28$^{-/-}$	Deficient in CD28, lacking T_{FH} and T_{FR} cells
	Icos$^{-/-}$	Deficient in ICOS, lacking T_{FH} and T_{FR} cells
	Tcra$^{-/-}$	Deficient in TCR α-chain, lacking T_{FH} and T_{FR} cells
	2D2	Transgenic mice, expressing a MOG-specific TCR and Foxp3-GFP
	Pdcd1$^{-/-}$2D2	With the properties of both *Pdcd1*$^{-/-}$ and 2D2 mice

Abbreviations: BLC, B lymphocyte chemoattractant; GC, germinal center; GFP, green fluorescent protein; PD, programmed death; PDCD-1, programmed cell death-1; TCR, T-cell receptor; Treg, regulatory T; WT, wild-type.

later. The isolated cells, in various combinations, were transferred into *Cd28*$^{-/-}$ mice and into TCR α-chain knockout (*Tcra*$^{-/-}$) mice. These mice cannot produce their own T_{FH} and T_{FR} cells, and so any follicular T cells in their bodies must have come from the transferred T_{FH} and T_{FR} cells. The recipients were then immunized with NP-OVA and the titers of NP-specific IgG were measured. In both recipient mice, the transferred WT T_{FH} cells promoted antibody production, whereas the transferred WT T_{FR} cells strongly inhibited the antibody production induced by the T_{FH} cells. Particularly, the transferred PD-1-deficient T_{FR} cells displayed much stronger suppressive ability than the WT T_{FR} cells. The T_{FR}-mediated inhibition depends on the 'follicular program' because CXCR5-deficient Treg cells failed to inhibit the antibody production induced by the WT T_{FH} cells. The T_{FR} cell-mediated inhibition was further confirmed by quantification of $CD138^+$ plasma cells in *Cd28*$^{-/-}$ and *Tcra*$^{-/-}$ mice after the transfer of WT T_{FH} and T_{FR} cells. These *in vivo* results demonstrate that T_{FR} cells in blood can home to lymph nodes and potently inhibit antibody production.

Taken together, the PD-1/PD-L1 pathway plays a critical role in T_{FR} generation and function. Signaling *via* PD-1/PD-L1 inhibits T_{FR} cell differentiation from $CD4^+Foxp3^+$ Treg cells and controls their suppressive ability, thus changing the balance between T_{FH} and T_{FR} cells. These findings provide new insights into the regulation of humoral immunity, and suggest that T_{FR} cells and associated molecules could be used as targets for the treatment of autoimmune diseases.

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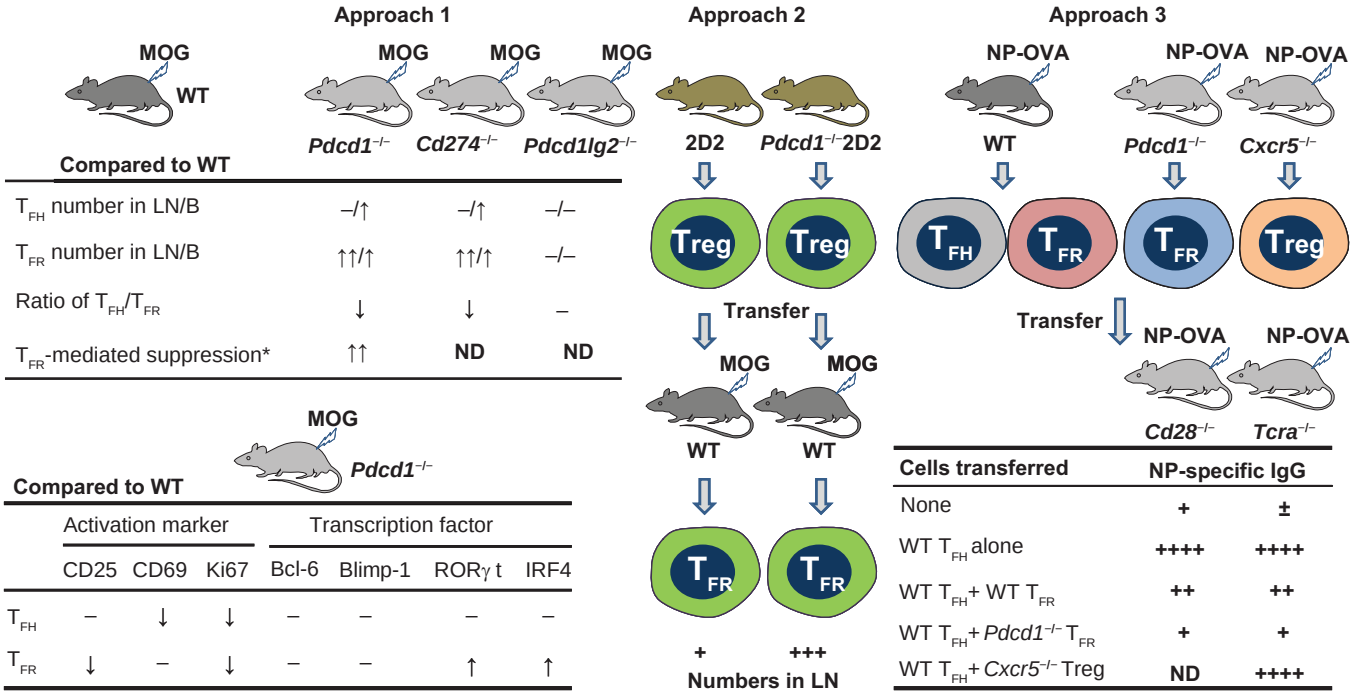

Figure 1 The major experimental approaches and findings which reveal the critical role of the PD-1/PD-L1 pathway in T_{FR} generation and function. *, T_{FR}-mediated suppression includes the inhibition of responder T- and B-cell proliferation, and the inhibition of IgG production. See Table 1 for the mice and antigens used. B, blood; LN, lymph nodes; MOG & NP-OVA, antigens; ND, not detected; PD-1, programmed death-1; –, no significant difference.

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