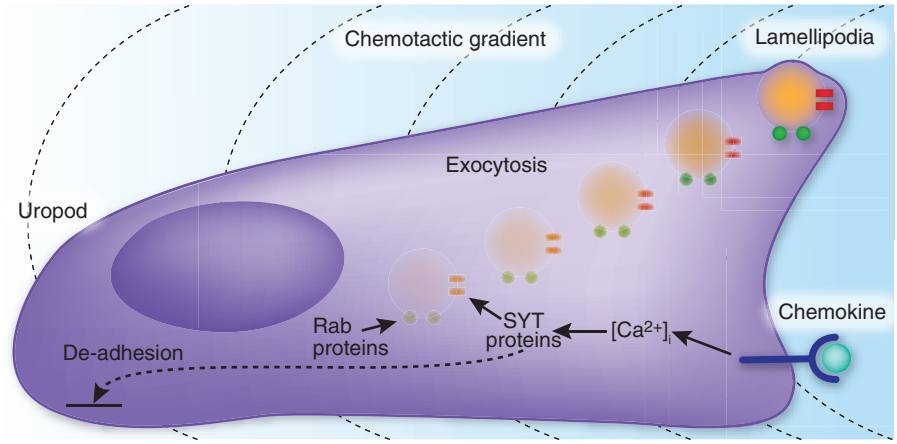

Figure 1 Leukocytes follow a chemotactic stimulus by sensing the gradient and generating a continuous cycling of adhesive events, cytoskeleton polymerization and plasma membrane remodeling. Colvin *et al.* now demonstrate the critical role of synaptotagmins (SYT proteins) and Rab small GTPases (Rab proteins) in controlling the permanent flow of preformed endomembranes to the plasma membrane, thus allowing continuous cell-shape changes. Chemokines trigger an increase in intracellular calcium ($[Ca^{2+}]_i$). This acts together with synaptotagmins and Rab small GTPases in controlling vesicle trafficking and exocytosis during chemotaxis. Exocytosis delivers to the cell surface the preformed layers of lipid membrane necessary for continuous plasma membrane turnover and extension toward the gradient. Synaptotagmins also participate in the adhesion–de-adhesion cycling that leads to uropod release.

chemotactic process itself by providing continuous delivery of preformed membrane layers critical to plasma membrane plasticity and that this trafficking of endomembranes critically regulates directional leukocyte motility.

The study raises questions that are surely important for future investigations. For example, the mechanism by which synaptotagmin-mediated vesicle fusion leads to rear release of uropods and chemotaxis is not known. Notably, to induce exocytosis, synaptotagmins interact with SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) proteins, which have a key role in vesicle and plasma membrane fusion by perturbing lipid bilayers[6]. Thus, SNARE proteins should be explored as possible mediators of membrane dynamics that control leukocyte rear release of uropods and locomotion. Moreover, the role of lysosome exocytosis should be investigated under different conditions, with the aim of possibly identifying different combinations of synaptotagmins and Rab isoforms that regulate chemotaxis in distinct leukocyte subtypes, thus introducing into the model important elements of cell specificity. Furthermore, it will be very useful to test whether the model also holds true for chemokinesis, which is probably more relevant to other cell contexts, such as metastatic cancer cells. In this last perspective, the study by Colvin and colleagues[2] may prove useful for envisioning novel directions for therapy.

COMPETING FINANCIAL INTERESTS
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Give and take in the germinal center

Stephen L Nutt & David M Tarlinton

B cell–T cell interactions in germinal centers are needed to generate high-affinity antibodies. PD-1 signaling is now shown to influence the quality of germinal center responses.

A major outcome of the immune response is the production of high-affinity antibody-secreting plasma cells and memory B cells. This process requires direct contact between activated B cells and T cells in a specialized structure known as the germinal center (GC). The GC supports cross-talk between B cells and T cells that controls their survival, proliferation and differentiation. In this issue of *Nature Immunology*, Shlomchik and colleagues demonstrate that the interactions between PD ligands on B cells and PD-1 on T cells is required for the optimal output of the GC; that is, long-lived antibody-secreting plasma cells[1].

PD-1, originally identified as a marker of programmed cell death, has subsequently been shown to be an important negative regulator of immune activation by the development of florid autoimmunity in PD-1-deficient mice[2]. More recently, PD-1 has come to signify a state known as immune exhaustion, in which T cells, unable to clear a viral infection, relapse into a torpor, apparently unable to raise the barest of effector functions[2]. Remarkably, however, blocking access to PD-L1—a ligand for PD-1—revives the exhausted T cells[3], which indicates that their inactive state is actively maintained. It is now widely held that PD-1 engagement promotes and maintains a state of T cell unresponsiveness.

PD-1 also has an alter ego, with high expression on T cells present in GCs[4]—so much so that PD-1, along with the costimulatory molecule ICOS and chemokine receptor CXCR5, has become a key means of identifying the $CD4^+$ follicular helper T cell (T_{FH} cell) subset (**Fig. 1**). Work on T_{FH} cells has exploded in recent years, particularly after the discovery that this population secretes interleukin 4 (IL-4) and IL-21 and is required for GC development[4]. This last connection is somewhat circular, as it has been clear for some time that there is an interdependence of the development of T_{FH}

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cells with that of GC B cells; each provides signals to the other that are essential for their development, persistence and function. The question now is what, how and when those signals are generated and transmitted.

One way of unraveling the interactions between B cells and T cells is to analyze immune responses that develop in the absence of specific molecules from either or both cell types (**Fig. 1**). Such an approach has identified CD40L, ICOS, Bcl-6 and IRF4 as being absolutely required for T_{FH} cell differentiation and thus GC development[4]. CXCR5, SAP, IL-21, IL-4 and various SLAM-family members are partially required in T cells, in that GCs develop in their absence but are defective in varying ways and to varying extents. The IL-21 receptor is not essential on T cells for T_{FH} cell or GC development but is required on B cells to sustain an initially normal GC reaction[5,6]. The defects in the GCs that form in the absence of various molecules on T_{FH} cells reflect either a failure to reach maximum size or premature termination. The fact that many of the ligands of the molecules needed to sustain the GC are expressed on B cells highlights the symbiotic nature of this B cell–T cell interaction; that is, T_{FH} cells require an array of signals from B cells to survive and to assume a fully functional state (**Fig. 1**), just as the maintenance of GC B cells requires episodic exposure to T_{FH} cells.

Deciphering the function of the individual components of the T cell–B cell interaction in the GC presents an ongoing challenge in immunology. To define the function(s) of the interactions of PD-1 with its ligands PD-L1 and PD-L2 in the GC, Good-Jacobson and colleagues have adopted a genetic approach and analyzed the immune response of mice deficient in PD-1, PD-L1 or PD-L2 or both ligands[1]. They find that although mice lacking either PD-1 or PD-L1 and PD-L2 initially have normal responses to immunization, the production of antibody-secreting cells is lower at later time points in all strains. This results from lower survival of PD-1-deficient GC B cells that is relatively specific for low-affinity antibody-secreting cells. Hence, PD signaling falls into the category of being a modulator of GC output rather than being essential for the process.

PD signals are also required for normal T_{FH} cell homeostasis, as mice deficient in PD-1 and those deficient in PD-L1 and PD-L2 have more T_{FH} cells[1]. Perhaps PD-1 ligation leads to the inhibition of T_{FH} cell proliferation in a manner similar to that previously found for other T cell lineages. Most interesting is that, despite the greater cell number, T_{FH} cells lacking PD molecules produce less of the two crucial cytokines known to provide help for GC cells: IL-4 and IL-21. Thus, PD-1 regulates the quantity and quality of T_{FH} cell responses to immunization.

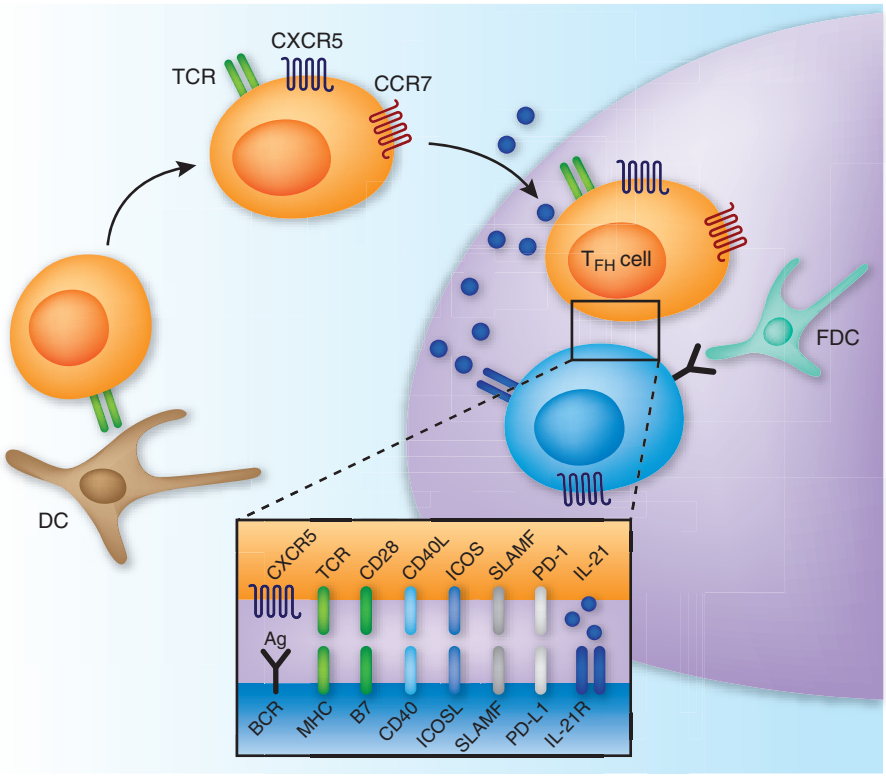

Figure 1 Important interactions between T_{FH} and B cells in the GC. Antigen-specific T cells, primed on dendritic cells (DC) in the T cell areas, upregulate ICOS, PD-1 and CXCR5 and migrate toward the B cell follicles. After interacting with their cognate B cells, these T cells mature into T_{FH} cells that express Bcl-6 and abundant PD-1. In the GC, T_{FH} cells interact with GC B cells through an array of molecular pairings, including pairings of T cell antigen receptor (TCR) and major histocompatibility complex (MHC) class II; CD28 and B7 family members; the costimulatory molecule CD40 and its ligand CD40L; ICOS and its ligand ICOSL; SLAM family members SLAMF on both cell types; and PD-1 and PD-L1. These interactions culminate in the secretion of cytokines by T cells, particularly IL-4 and IL-21, which are received by the B cells to influence their subsequent activity. CCR7, chemokine receptor; FDC, follicular dendritic cell; Ag, antigen; BCR, B cell antigen receptor; IL-21R, IL-21 receptor.

As the PD proteins are expressed by many cell types, a critical question is the identity of the particular cell types responsible for the observed defects. Good-Jacobson and colleagues address this question by using a variety of cell-transfer and chimeric approaches that support the general conclusion that expression of PD-L1 and PD-L2 on B cells and expression of PD-1 on T cells regulate both plasma cell production and some aspects of memory B cell formation[1]. These studies, however, do not rule out the possibility of some roles for other interactions, such as PD-1–PD-L1 and PD-1–PD-L2 interactions between B cells (discussed below).

The identification of PD-1 as an important participant that promotes the output of the GC stands in stark contrast to the well-established function of PD-1 as an inhibitor of T cell activation and immune pathology[2]. Although T_{FH} cells may simply be different from other T cells in their response to PD-1-derived signals, another possibility is that T_{FH} cells require this inhibitory signal to focus their full attention to the job of helping GC B cells. The lower production of IL-4 and IL-21 by T_{FH} cells that lack PD-1, despite the greater prevalence of T_{FH} cells, would support this possibility, particularly because the GC phenotype reported here and the GC phenotype that arises in the absence of IL-21 signaling are strikingly similar. More work is needed to tease out how PD-1 signals affect T_{FH} cells on a cellular and molecular level.

Another aspect of PD biology still not fully understood is the role of B cell–B cell signals or reverse B cell–T cell signals (in which PD-1 is expressed on B cells). Although Good-Jacobson and colleagues demonstrate that PD-1 on T cells and PD-L1 and PD-L2 on B cells represent the crucial interaction for the production of long-lived plasma cells[1], the data leave open the possibility that B cell–B cell or B cell–T cell signals are important for memory B cells. Given the large excess of B cells relative

to T_{FH} cells in a GC, this possibility requires future consideration.

Finally, why PD deficiency affects only late GC reactions remains to be fully explained. PD molecules are induced rapidly after activation of B cells and T cells, yet the defect in plasma cell production occurs in the second half of the lifespan of the GC. Good-Jacobson and colleagues propose that the defect represents the cumulative result of smaller quantitative changes in survival and differentiation over multiple divisions[1]. An alternative possibility is that PD signals may have a distinct and unknown stage-specific function in late GC, a function that may also involve IL-21 (ref. 6).

It is reasonable to ask whether there is now sufficient information to explain how T cell–B cell interactions in the GC achieve the outcomes associated with productive immune responses: affinity maturation, memory B cells and long-lived plasma cells. CXCR5 is important but not essential for locating T cells in the GC and allowing them to reach their full maturity[7]; interactions between SLAM family members extend the duration of cell-cell interactions and may have this role in the GC[8]; and the role of Bcl-6 in T_{FH} cells remain unknown[4]. It is possible that the signals delivered through PD-1 maintain T_{FH} cells in an active state, particularly their secretion of IL-21, and thereby maintaining B cell proliferation through sustained expression of Bcl-6, with all the qualitative and quantitative consequences this has for B cell memory[5,6]. Finally, the number and range of molecules involved in the T cell–B cell interaction in the GC may seem excessive at first inspection. Some, such as CD40, are clearly essential, whereas many others have more subtle roles. It may be that each molecular pairing has a specialized function, as suggested by Good-Jacobson and colleagues for PD-1 in plasma cell formation[1], for example. An alternative is that certain, possibly essential, functions of some molecules are obscured by immunization strategies that maximize responses yet may be demonstrated in more physiological settings. Time and further experimentation will tell.

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Catenin' on to nucleic acid sensing

Vijay A K Rathinam, Shruti Sharma & Katherine A Fitzgerald

Many pathogens induce a type I interferon response via a pathway dependent on the kinase TBK1 and transcription factor IRF3. However, LRRFIP1, a cytosolic sensor of DNA and RNA, triggers interferon production by a β-catenin-dependent signal.

The activation of innate immunity requires the integration of complex networks of signal-transduction pathways, which leads to the induction of specific sets of genes in response to a wide range of infectious microorganisms[1]. Specificity is achieved by the coordinated activation of multiple transcription factors that assemble on enhancers located upstream of the target genes. A well-characterized example of this is the human gene encoding interferon-β (*IFNB1*). Transcription of *IFNB1* is tightly regulated and is induced in a highly ordered process that involves in most cases activation of the transcription factors NF-κB and IRF3 and the heterodimeric transcription factor complex ATF-2–c-Jun. These factors bind cooperatively to the *IFNB1* enhancer and recruit coactivators such as CBP and p300 and chromatin-remodeling proteins to the *IFNB1* promoter. In this issue of *Nature Immunology*, Yang *et al.* demonstrate that the cytosolic nucleic acid–binding protein LRRFIP1 and its downstream signal transducer β-catenin are critical components of a coactivator pathway that regulates transcription of the gene encoding interferon-β (IFN-β) during infection with *Listeria monocytogenes* or vesicular stomatitis virus (VSV)[2].

Although NF-κB and ATF2–c-Jun regulate a wide range of immune-response genes, the interferon-regulatory factors are selectively required for transcription of *IFNB1*. IRF3 is the best studied of these and is a critical component of the *IFNB1* enhanceosome. IRF3 normally resides in the cytosol of cells and must be phosphorylated to dimerize, translocate to the nucleus and control *IFNB1* transcription. IRF3 is phosphorylated by the IκB kinase–related kinase TBK1 (also called NAK or T2K). The molecular mechanisms that lead from the recognition of microbial pathogens such as *L. monocytogenes* to signal-dependent phosphorylation of IRF3 have been the subject of intense investigation over the past decade or more[3]. Several classes of germline-encoded pattern-recognition receptors have been shown to sense microbial products and trigger signaling pathways that lead to IRF3 phosphorylation[4]. These include Toll-like receptor 3 (TLR3) and TLR4 (which sense double-stranded RNA and lipopolysaccharide, respectively), as well as the RNA helicases RIG-I and Mda5, which discriminate between different classes of RNA viruses. Growing evidence also indicates the importance of cytosolic DNA–sensing mechanisms in the IFN-β response to *L. monocytogenes*, as well as to a wide range of additional bacterial, viral and parasitic infections. These pathways are complex and seem to involve multiple receptors that converge on the endoplasmic reticulum–resident protein STING, which acts upstream of TBK1 and IRF3. DAI has been found to bind double-stranded DNA (dsDNA) from various sources and induce the production of IFN-β. In addition, RNA polymerase III converts AT-rich dsDNA into an RNA intermediate that subsequently triggers IFN-β via RIG-I. This last pathway is critical in human cells for a select subset of pathogens but seems to be redundant with additional mechanisms upstream of STING in mouse macrophages and dendritic cells[5].

In an effort to identify a receptor and/or previously unknown components of cytosolic DNA–sensing pathways, Yang *et al.* use a small interfering RNA (siRNA) screening approach to target the *L. monocytogenes*–induced IFN-β

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