identified by Schnyder et al. in B cells play an analogous role in T cells. And in both systems, it will be important to continue to define the molecular mechanisms that control microcluster movement along the microtubule network. But there are also broader questions. The implication from these papers is that signaling microclusters generated in an actin-dependent fashion in the periphery of the IS and moved initially by actomyosin-dependent forces transition to dynein-dependent movement for final delivery into the center of the IS. If so, how does this "hand-off" take place without an obvious change in microcluster velocity or trajectory, and why does depolymerization of actin filaments result in loss of microcluster movement, rather than enhanced dynein-dependent movement? How does the microtubule network contribute to the supramolecular segregation of signaling components? Clearly, current models invoking differential actin binding or actin-dependent clustering (Hartman et al., 2009) must be revised. How do the two filament systems work with respect to plasma membrane-associated proteins versus vesicle-associated molecules? And finally, how are the actin and microtubule networks coordinated? Based on studies in nonhematopoietic cells (Etienne-Manneville, 2004), it seems likely that regulation of these two scaffolding systems is intertwined, and that understanding this crosstalk will be essential for understanding cytoskeletal control of lymphocyte function.

REFERENCES

Burkhardt, J.K., Carrizosa, E., and Shaffer, M.H. (2008). Annu. Rev. Immunol. *26*, 233–259.

Etienne-Manneville, S. (2004). Traffic *5*, 470–477.

Hartman, N.C., Nye, J.A., and Groves, J.T. (2009). Proc. Natl. Acad. Sci. USA *106*, 12729–12734.

Harwood, N.E., and Batista, F.D. (2010). Annu. Rev. Immunol. *28*, 185–210.

Hashimoto-Tane, A., Yokosuka, T., Sakata-Sogawa, K., Sakuma, M., Ishihara, C., Tokunaga, M., and Saito, T. (2011). Immunity *34*, this issue, 919–931.

Huse, M., Quann, E.J., and Davis, M.M. (2008). Nat. Immunol. *9*, 1105–1111.

Kupfer, A., and Singer, S.J. (1989). Annu. Rev. Immunol. *7*, 309–337.

Lasserre, R., and Alcover, A. (2010). FEBS Lett. *584*, 4845–4850.

Lasserre, R., Charrin, S., Cuche, C., Danckaert, A., Thoulouze, M.I., de Chaumont, F., Duong, T., Perrault, N., Varin-Blank, N., Olivo-Marin, J.C., et al. (2010). EMBO J. *29*, 2301–2314.

Schnyder, T., Castello, A., Feest, C., Harwood, N.E., Oellerich, T., Urlaub, H., Engelke, M., Wienands, J., Bruckbauer, A., and Batista, F.D. (2011). Immunity *34*, this issue, 905–918.

Serrador, J.M., Cabrero, J.R., Sancho, D., Mittelbrunn, M., Urzainqui, A., and Sanchez-Madrid, F. (2004). Immunity *20*, 417–428.

A Fine Romance: T Follicular Helper Cells and B Cells

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T follicular helper (Tfh) cells help B cells to generate affinity-matured antibodies. Three papers in this issue of *Immunity* (Choi et al., 2011; Kerfoot et al., 2011; Kitano et al., 2011) provide information about the reciprocal relationship between B cells and Tfh cells.

It was reported more than two decades ago that T cell clonal expansion in lymph nodes (LN) was impaired in mice made deficient in B cells by continuous injections of antibodies directed against the heavy chain from birth (Ron and Sprent, 1987). Like other T cells, B cells are crucial for the development of a specialized subset of $CD4^+$ T helper cells known as T follicular helper (Tfh) cells (Haynes et al., 2007). The relationship between Tfh cells and B cells is thought to be especially important because of a reciprocal dependency played out during the generation of affinity-matured antibody. This cognate interaction occurs in specialized, temporary structures, termed germinal center (GC) reactions, that form within B cell follicles of secondary lymphoid organs after infection or immunization with nonreplicating T cell-dependent antigen.

GC B cells require T cell help to produce affinity-matured antibody. More recently, however, evidence has been presented to show that this dependence is not reciprocal because Tfh cells can develop without B cells, provided that the T cells get adequate stimulation from peptide antigen-MHCII complexes displayed on other antigen-presenting cells (APCs) (Deenick et al., 2010). This finding indicated that the role of B cells may reflect their ability to provide an ample source of antigen to Tfh cells and questioned whether B cells provide any unique signals. Which antigen-presenting cells Tfh cells interact with at different points during their activation is explored in detail in three papers in the current issue of *Immunity* (Choi et al., 2011; Kerfoot et al., 2011; Kitano et al., 2011). One clear

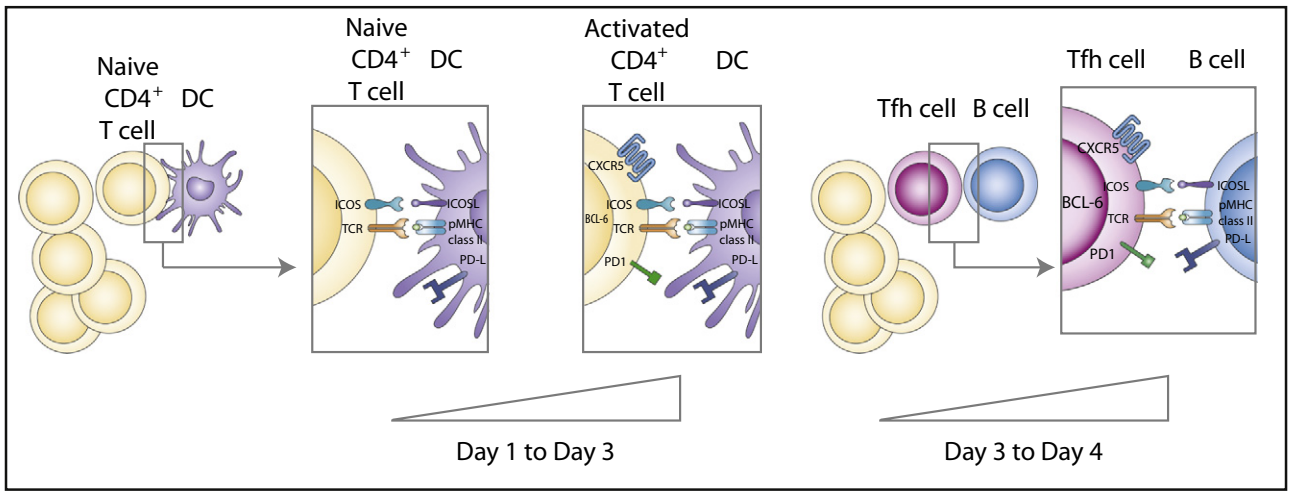

Figure 1. Differentiation and Maintenance of Follicular Helper T Cells by Antigen-Presenting Cells

Upon activation of naive CD4$^+$ T cells by peptide MHC class II complexes on antigen-presenting cells (APCs) (most probably dendritic cells [DCs]), inducible T cell costimulator (ICOS), programmed cell death 1 (PD-1), the transcriptional repressor B cell lymphoma 6 (Bcl-6), and the chemokine receptor CXCR5 are upregulated within the first 3 days of exposure to antigen. The costimulatory molecule ICOS and coinhibitory molecule PD-1 expressed on CD4$^+$ T cells are the receptors for ICOSL and PD-L1 (or PD-L2) expressed on APCs. By day 4, antigen-experienced B cells become crucial APCs for T helper cells and the cognate interaction with B cells sustain T follicular helper (Tfh) cells with high expression of Bcl-6 and high surface density of ICOS, PD-1, and CXCR5.

consensus is that although B cells are dispensable for the priming of CD4$^+$ T cells and initial acquisition of a Tfh cell phenotype, they are important for the maintenance and function of Tfh cells during the GC reaction (Figure 1).

T and B cells are distinctly compartmentalized within secondary lymphoid tissues and dynamic changes in lymphocyte migration occur during immune responses that facilitate the chance of contact between rare antigen-experienced B cells and T helper cells. Placing Tfh cells in an in vivo context via immunohistology and intravital approaches, Kitano et al. (2011) and Kerfoot et al. (2011) visualized early events in the B cell follicle and intrafollicular (IF) zone providing important temporal and spatial information about the early progression toward a Tfh cell phenotype and a GC B cell phenotype. Several recent studies have shown that macrophages positioned at the subcapsular sinus (SCS) acquire antigen that is presented to B cells that then migrate into the B cell follicle (Cyster, 2010). However, in the study by Kerfoot et al. (2011), a trend of movement of B cells from the SCS into the follicular interior was not discerned, and whether this reflected the form of antigen or other aspects of the experimental system will be the subject of future studies.

Characteristically, Tfh cells express high amounts of the CXC-chemokine receptor 5 (CXCR5) on their surfaces and lose expression of CCR7, which directs these cells toward CXCL13-rich follicular dendritic cells within GC. However, previous studies have shown that the expression of CXCR5 is transiently upregulated when CD4$^+$ T cells interact with peptide-MHC class II and costimulatory molecules on APCs (King et al., 2008). Similarly, the expression of the inducible T cell costimulator (ICOS) and the coinhibitory molecule, programmed cell death 1 (PD-1), both used to detect Tfh cells, are common to recently activated CD4$^+$ T cells. Examination of the kinetics of expression of these molecules extends the list of Tfh cell markers that are broadly expressed on CD4$^+$ T cells within 3 days of antigen administration to include GL7 and the transcriptional repressor B cell lymphoma 6 (Bcl-6) (Choi et al., 2011; Kerfoot et al., 2011; Kitano et al., 2011). This information, gained from detailed kinetic analyses of the development of Tfh cells during immune responses, demonstrates that the initial interaction of CD4$^+$ T cells with dendritic cells provides the stimulus for the upregulation of CXCR5, ICOS, PD-1, GL7, and Bcl-6 (Figure 1; Choi et al., 2011; Kerfoot et al., 2011; Kitano et al., 2011). Indeed, these "Tfh cell phenotypic markers" described the majority of antigen-specific CD4$^+$ T cells 2 days after immunization (Kerfoot et al., 2011). By contrast, 8 days after immunization, in the mature GC, PD-1^{hi}CXCR5^{hi} Tfh cells were clearly distinguished (Kerfoot et al., 2011).

In each of these studies, the stimulus required to sustain these molecules on CD4$^+$ T cells was provided by cognate interactions with B cells (Choi et al., 2011; Kerfoot et al., 2011; Kitano et al., 2011). Indeed, by day 4 of immunization in the absence of cognate B cells, cells expressing a Tfh cell phenotype were lost (Choi et al., 2011). Although there were markedly less Tfh phenotype cells in the absence of B cells, they did migrate into the B cell follicle (Kerfoot et al., 2011). This observation begs the question of whether there was a functional difference between Tfh cells that develop in the presence or absence of B cells. Importantly, Tfh cells that developed in the absence of B cells were functionally impaired with reduced IL-4 and IL-21 (Kerfoot et al., 2011). One caveat (noted by the investigators) is that the use of B cells with a fixed BCR receptor affinity toward the antigen and antigen-specific TCR transgenic T cells might influence the kinetics and fate commitments of the response (Choi et al., 2011; Kerfoot et al., 2011; Kitano et al., 2011). It was therefore satisfying to observe that the expression of Tfh cell molecules similarly emerged 3 days after LCMV infection (Choi et al., 2011).

Tfh cells require strong signals through the antigen receptor and costimulation in the form of cell surface molecules and cytokine growth factors (King et al., 2008). However, increased responsiveness to IL-2 does not appear to be crucial for Tfh cell differentiation or survival as evidenced by the segregated expression of Bcl-6 and the α chain of the high-affinity IL-2 receptor (Choi et al., 2011). Whether this reinforces their differential expression of the B lymphocyte-induced maturation protein 1 (Blimp) and Bcl-6 remains to be determined. The mechanisms explaining the regulation of Bcl-6 remain largely unknown, but the induction of Bcl-6 in $CD4^+$ T cells requires ICOS (Choi et al., 2011). Furthermore, ICOS induction of Bcl-6 expression was observed to sequentially induce CXCR5 expression prior to the involvement of B cells (Choi et al., 2011). Utilizing a reporter mouse strain generated to track Bcl-6 protein expression in vivo, Kitano et al. (2011) observed that upregulation of Bcl-6 protein in antigen-engaged $CD4^+$ T cells preceded that of antigen-engaged B cells. Marked heterogeneity was observed in the expression of Bcl-6 in Tfh phenotype cells (Kerfoot et al., 2011; Kitano et al., 2011), yet these new studies concur with previous studies showing that $CD4^+$ T cells further upregulated Bcl-6 expression through cognate interactions with B cells (Poholek et al., 2010).

Interestingly, Bcl-6 protein was gradually lost in Tfh cells whereas Bcl-6 protein was maintained in GC B cells (Kitano et al., 2011). After downregulation of Bcl-6, Tfh cells decreased proliferation and increased expression of the α chain of the receptor for IL-7. However, despite the loss of Bcl-6 protein, the expression of the gene for Blimp1, positive regulatory domain I (*Prdm1*), remained low in Bcl-6^{lo} Tfh cells and mRNA transcript for Bcl-6 remained high (Kitano et al., 2011). The basis for these interesting findings will require clarification and the influence of one potentially defective *Bcl6* allele in the heterozygous reporter cells remains unknown. Extended temporal analyses will be needed to analyze the population of Bcl-6^{lo} Tfh cells. Whether the Tfh cells that downregulate Bcl-6 have the potential to redirect their differentiation into other Th cell subsets, stay in the GC as memory phenotype Tfh cells (Fazilleau et al., 2007), or join the circulating pool of $CXCR5^+CD4^+$ memory T cells will be the focus of future studies.

Thus, Tfh cells uniquely receive continued stimulation through their antigen receptors beyond the time when effector $CD4^+$ T cells have migrated out of the lymphoid organ and the local $CD4^+$ T cell response has begun to contract. In this context, Tfh cells appear to become transfixed in an activated phenotype maintained by high expression of the transcriptional repressor Bcl-6, which is consistent with their original description as an activated, nonpolarized subset (King et al., 2008). Like all interesting work, these recent studies raise many questions. The term "maintenance" of Tfh cells is used extensively in these three studies (Choi et al., 2011; Kerfoot et al., 2011; Kitano et al., 2011), but what maintenance actually means remains enigmatic. B cells may be important for the continued clonal expansion of Tfh phenotype cells, as shown by the proliferation of Bcl-6-expressing T cells in the presence of cognate B cells (Kitano et al., 2011), or B cells may be required to sustain features of the Tfh cell phenotype. Alternatively, B cells may be important for the survival of Tfh cells—a possibility that is supported by the increased numbers of Tfh cells observed within B cell follicles in the presence of cognate B cells on days 4 and 8 after immunization (Kerfoot et al., 2011). Efforts to define the mechanism(s) explaining how B cells support Tfh cells will continue to generate research within this exciting field. Collectively, these new studies provide a kinetic framework for Tfh cell development that advance our understanding of immune responses and will be valuable for improved vaccine design.

REFERENCES

Choi, Y.S., Kageyama, R., Eto, D., Escobar, T.C., Johnston, R.J., Monticelli, L., Lao, C., and Crotty, S. (2011). Immunity *34*, this issue, 932–946.

Cyster, J.G. (2010). Nat. Immunol. *11*, 989–996.

Deenick, E.K., Chan, A., Ma, C.S., Gatto, D., Schwartzberg, P.L., Brink, R., and Tangye, S.G. (2010). Immunity *33*, 241–253.

Fazilleau, N., Eisenbraun, M.D., Malherbe, L., Ebright, J.N., Pogue-Caley, R.R., McHeyzer-Williams, L.J., and McHeyzer-Williams, M.G. (2007). Nat. Immunol. *8*, 753–761.

Haynes, N.M., Allen, C.D., Lesley, R., Ansel, K.M., Killeen, N., and Cyster, J.G. (2007). J. Immunol. *179*, 5099–5108.

Kerfoot, S.M., Yaari, G., Patel, J.R., Johnson, K.L., Gonzalez, D.G., Kleinstein, S.H., and Haberman, A.M. (2011). Immunity *34*, this issue, 947–960.

King, C., Tangye, S.G., and Mackay, C.R. (2008). Annu. Rev. Immunol. *26*, 741–766.

Kitano, M., Moriyama, S., Ando, Y., Hikida, M., Mori, Y., Kurosaki, T., and Okada, T. (2011). Immunity *34*, this issue, 961–972.

Poholek, A.C., Hansen, K., Hernandez, S.G., Eto, D., Chandele, A., Weinstein, J.S., Dong, X., Odegard, J.M., Kaech, S.M., Dent, A.L., et al. (2010). J. Immunol. *185*, 313–326.

Ron, Y., and Sprent, J. (1987). J. Immunol. *138*, 2848–2856.