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Taking Control of Follicular Dendritic Cells

Follicular dendritic cells signal the formation of B cell follicles as well as B cell activation, proliferation, and maturation. However, the precise molecular pathways controlling these processes are just beginning to be unraveled.

Establishment of T cell-dependent humoral immunity requires key cell-cell interactions in the B cell follicles of secondary and tertiary lymphoid tissues. Clusters of follicular dendritic cells (FDCs) provide both organizing signals for the proper structure of the follicles and regulatory signals to support B cell activation, proliferation, and maturation. In this issue of *Immunity*, Victoratos et al. underscore the requirement for tumor necrosis factor (TNF) to activate FDCs and identify IkappaB kinase 2 (IKK2)-dependent signaling as critical for the expression of a fully functional germinal center B cell response (Victoratos et al., 2006).

Properly regulated immune responses, which include development of robust immune memory, require interactions between antigen-presenting cells (APCs), effector lymphocytes, and additional regulatory cells. Efficient induction of T cell-dependent immune responses occurs largely in secondary and tertiary lymphoid tissues (Karrer et al., 1997). These lymphoid tissues provide an organized environment in which naive antigen-specific cells and antigen-nonspecific accessory cells and regulatory cells accumulate in preparation for an encounter with antigen. Delivery of antigens into a lymphoid organ causes antigen-specific T cells and B cells to be activated and to move to new locations that facilitate their interactions to generate high-affinity antibody responses and durable immune memory.

The lymphoid tissue compartment designated the B cell follicle is central in the formation of high-affinity antibody responses and B cell memory. In normal lymphoid organs, it is the site where the germinal center (GC) reaction develops. Although immunoglobulin gene somatic hypermutation and selection of high-affinity antibody can occur without GCs (Matsumoto et al., 1996), intact GCs dramatically enhance the efficiency of the high-affinity response (Le Hir et al., 1996). In lymphoid tissues, mature FDCs play key roles as follicle organizers. When they receive the proper developmental and maturational signals, FDCs cluster and express the chemokine CXCL13 and adhesion molecules that help localize cells that participate in the GC response. Once they are clustered, FDCs express a dense network of dendritic processes that display both complement and Fc receptors that localize antigen-containing immune complexes required for the efficient generation and selection of high-affinity, somatically mutated antibodies and for the formation of memory B cells. In addition to binding immune complexes, FDCs interact avidly with B cells by virtue of the adhesion molecules ICAM-1 and VCAM-1 on the FDCs, which interact with LFA-1 and VLA-4 on the B cell. These physical interactions are crucial for the survival of B cells proliferating in the GC (Koopman et al., 1994; Tew et al., 2001). In addition to supporting survival of GC B cells, FDCs appear to activate both isotype switching and somatic hypermutation, inducing expression of the activation-induced cytidine deaminase (AID) in B cells that have been stimulated with antigen (Aydar et al., 2005).

Considerable progress has been made in defining signals that affect FDC differentiation and maturation. Many studies have shown that FDCs are derived from radio-resistant, nonhematopoietic lineage cells. The exact nature of these precursor cells remains undefined. Nevertheless, it is clear that interactions between several ligands in the TNF family and their receptors are required for normal development of FDCs from these precursors and for normal FDC survival and function. Mice deficient in the membrane lymphotoxin heterotrimer ($LT\alpha_1\beta_2$) or its cell-surface receptor ($LT\beta R$) manifest congenital absence of lymph nodes and Peyer's patches as well as disordered spleen structure with absence of detectable FDCs (Matsumoto et al., 1996). A direct role for $LT\alpha_1\beta_2$ in FDC differentiation or activation is supported

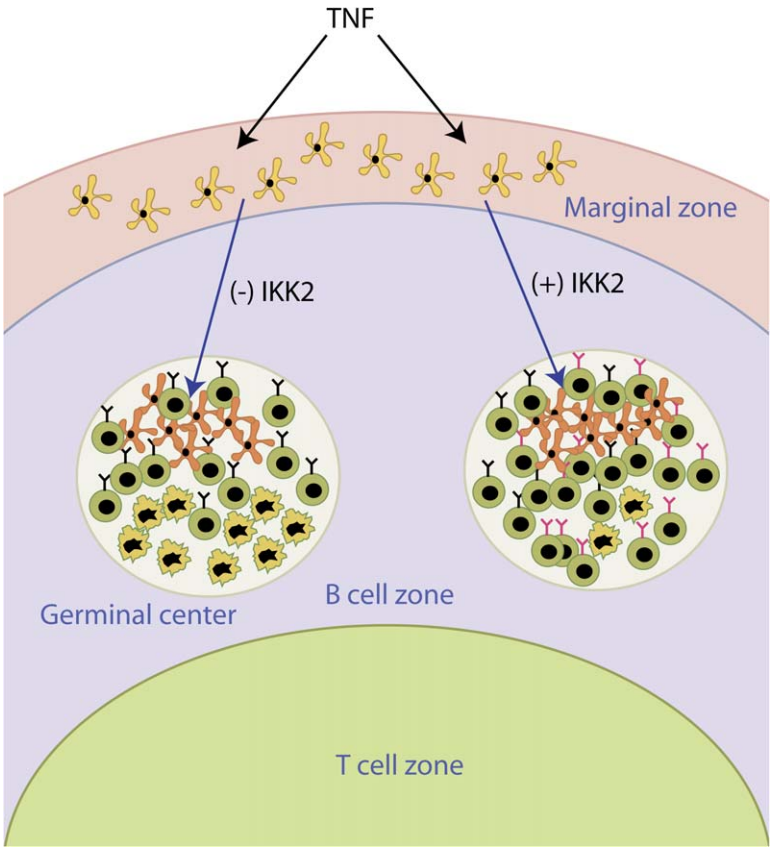

Figure 1. TNF Signals Induce Formation of Clustered FDC and with IKK2 Support Functional GC B Cell Responses

In TNF-deficient mice, cells bearing FDC surface markers accumulate abnormally in the spleen white pulp marginal zone. Restoration of TNF signaling leads to relocation of these FDCs or immature FDC precursors to nascent B cell follicles where the cells cluster and form a rich dendritic network. Signaling through the p55TNFR leads to activation of NF-κB transcription factors via two pathways. The classical pathway that includes signaling by IKK2 induces B cell resistance to apoptosis, AID gene expression, isotype switching, and affinity maturation. In the absence of IKK2, clusters of FDCs form that are capable of supporting GC B cell proliferation but that poorly support isotype switching, affinity maturation, and B cell survival.

by the observations that FDCs bear LTβR on their surfaces and that treatment of mice with soluble LTβR resulted in disappearance over several days of detectable FDCs (Mackay and Browning, 1998). This latter study could not, however, exclude the possibility that the effect of $LT\alpha_1\beta_2$-LTβR signaling was mediated indirectly through a non-FDC cell that provided a trophic signal required by the FDC.

Mice deficient in TNF or in the p55 isoform of the TNF receptor (p55TNFR) also show disturbed FDC maturation (Le Hir et al., 1996). In this case, however, cells bearing FDC-specific surface markers are found in the spleen in large numbers, but inappropriately localized in the marginal zone (Pasparakis et al., 2000). Furthermore, the adjacent B cells, without signals from clustered FDCs, show no detectable follicular organization. GCs do not form after immunization, and antibody responses and B cell memory are profoundly suppressed. It is not known whether these marginal zone FDC-like cells are FDC precursors or are incompletely activated or differentiated FDCs. Interestingly, treatment of TNF-deficient mice with an activating p55TNFR monoclonal antibody caused rapid clustering of FDCs with the formation of a rich dendritic network and restoration of the ability to form GCs (Mandik-Nayak et al., 2001). In vivo, the TNF signal that leads to FDC clustering and maturation is thought to be delivered by follicular B cells, and thus represents an example where the B cell provides signals that support the development of a specialized microenvironment in which the B cells themselves can express their mature functions. Although FDCs bear p55TNFR on their surfaces, studies performed to date have not determined whether TNF acts directly on the FDC to induce their relocalization from the marginal zone into clusters within the follicular area (Figure 1). In fact, it is not clear whether TNF signaling occurs solely within the marginal zone to permit transition of the FDCs from this compartment into the follicular areas or whether it acts at multiple locations within the white pulp.

Victoratos et al. have taken an elegant approach to address the question of whether p55TNFR acts in a cell-autonomous fashion for the maturation of FDCs (Victoratos et al., 2006). Their strategy takes advantage of the tissue specificity of CD21 (complement receptor 2) expression to drive lineage-specific reactivation of a *Tnfr1a* (encoding p55TNFR) null mutation via Cre-loxP recombination intended to affect only FDCs. The *Tnfr1a*-null mutation was created by inserting a neomycin-resistance cassette flanked by upstream and downstream loxP sites into an intron of the *Tnfr1a* gene in a fashion that completely interrupted expression of the p55TNFR. Expression of Cre under the CD21 promoter excises the *neo* cassette and restores p55TNFR expression only in cells that express CD21. Because CD21 expression is high in mature B cells and in FDCs, this strategy results in mice with restored p55TNFR in mature B cells and FDCs. To eliminate the contribution of B cell-derived p55TNFR, CD21-reactivated mice were lethally irradiated and transplanted with bone marrow from $Tnfr1a^{-/-}$ mice, replacing the recipient's $p55TNFR^{+}$ B cells with donor p55TNFR-deficient B cells, leaving

p55TNFR expressed in the CD21$^+$ radio-resistant recipient FDCs. Mice treated in this fashion showed restoration of FDC clustering in the spleen and lymph nodes and parallel restoration of GC function, excluding a requirement for p55TNFR on B cells in the activation of FDC clustering and FDC function. CD21 is also known to be expressed, albeit at lower amounts, in subsets of immature thymocytes and in several epithelial tissues (Timens et al., 1991). It is, thus, difficult to exclude completely a contribution of radio-resistant CD21$^+$ non-FDC to the restoration of normal FDC localization and function. Such cells could be present and active in the mature lymphoid organs or might be developmentally regulated and absent from the mature tissue. With this caveat, the studies reported here provide the strongest evidence to date that TNF acts directly on FDC to induce their clustering within the B cell follicle and to activate their ability to support a functional GC response. It will be of interest to extend this model to determine whether the FDC clusters that form in tertiary lymphoid nodules at sites of chronic inflammation, which may be important in the tissue destruction associated with this inflammation, are governed by the same TNF stimuli.

Victoratos et al. used a similar approach to investigate postreceptor signaling in the restoration of FDC clustering and function. Previous studies have shown that intracellular signaling molecules of the nuclear factor-κB (NF-κB) family mediate p55TNFR-induced activation of FDC. The classical NF-κB signaling pathway acts through IKK2 and causes nuclear translocation of p50:RelA heterodimers to activate genes involved in inflammation and innate immune responses, whereas the alternative NF-κB pathway acts through the other kinases IKK1 and NIK and causes nuclear translocation of p52:RelB heterodimers to regulate genes that mediate lymphotoxin-induced lymphoid organogenesis and lymphoid tissue homeostasis (Figure 1). Mice with IKK2 deficiency only in the radio-resistant FDCs were prepared by CD21-Cre-driven targeting to ablate IKK2 expression in CD21$^+$ cells followed by reconstitution of IKK2 expression in B cells by transplantation with normal bone marrow. These mice showed that IKK2-deficient FDCs clustered in a fashion indistinguishable from wild-type FDCs and also supported the formation of morphologically normal GC. This observation suggests that the classical NF-κB pathway is not required for proper localization and clustering of FDCs. In contrast, although IKK2-deficient FDC could bind and retain immune complexes in an apparently normal fashion, they were unable to upregulate their cell-surface expression of ICAM-1 and VCAM-1 and could not support the upregulation of AID and other activation-associated factors in GC B cells (Victoratos et al., 2006). This defect was associated with increased GC B cell apoptosis and failure to generate a high-affinity, isotype-switched antibody response. Thus, in FDCs, activation of the p55TNFR induces IKK2-independent signals that support FDC clustering, localizing of immune complexes, and activation of B cell proliferation to form a morphological GC. Activation of the p55TNFR also induces IKK2-dependent signals that support upregulation of ICAM-1 and VCAM-1 expression, suppression of GC B cell apoptosis, and expression of high-affinity antibodies. With their dependence on the upregulation of ICAM-1 and VCAM-1 expression, these activities may represent p55TNFR-induced quasi-inflammatory responses, explaining their IKK2 dependence. Future experiments analyzing the gene-expression profiles of IKK2-expressing and IKK2-deficient FDCs will identify key genes involved in the activation and function of these cells in the GC response. Additional experiments in which the expression of ICAM-1 and VCAM-1 is artificially maintained in the absence of IKK2 expression may permit determination of the role of cell adhesion as a driver of a productive GC reaction.

The experiments reported by Victoratos et al. have unequivocally established FDCs as critical cells organizing the B cell follicle and the mature GC response. They also provide proof of principle that the strategies of lineage-specific gene targeting and lineage-specific gene repair can dissect complex issues of tissue function that are not accessible to standard biochemical and molecular techniques.

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