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Follicular dendritic cell networks of primary follicles and germinal centers: phenotype and function

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Abstract

Follicular dendritic cells (FDCs) were identified decades ago by their ability to retain immune complexes and more recent findings indicate that they are a source of B cell attractants and trophic factors. New imaging studies have shown that B cells closely associate with their dendritic processes during migration. Here we will review the properties of these specialized follicular stromal cells and provide an update on the requirements for their maturation into phenotypically distinct cells within germinal center light and dark zones. We will then discuss current understanding of how they help support the B cell immune response.

Keywords

antibody response; lymphocyte; FDC; germinal center; lymph node; stromal cell; immune complex

1. Introduction

B cell follicles in lymphoid organs can exist in either of two states: 1) quiescent (primary) follicles, which are composed largely of naïve B cells migrating extensively in search of cognate antigen and 2) activated (secondary) follicles, which contain a central germinal center (GC) full of B cell blasts undergoing events associated with antibody affinity maturation. While primary and secondary follicles are both B cell rich environments, they support distinct B cell populations and thus have unique stromal requirements.

Many of the stromal cells in B cell follicles and also in T zones are associated with the tissue-supporting network of branched, collagenous fibers and extracellular matrix molecules that they generate. This network of fibers is also known as a reticulum (reticulum derives from the Latin word *rete* meaning net), and the stromal cells that ensheath these fibers were historically referred to as reticular cells. The reticular cells in B cell follicles, also termed follicular stromal cells, have been extensively described by ultrastructural studies (reviewed in [1,2]).

Within the light zones of GCs, distinct reticular cells were observed compared with the neighboring follicular mantle zones and these were termed dendritic reticular cells. These cells extended long cytoplasmic processes but did not ensheath collagenous fibers. Ultrastructural studies suggested that these cells might represent a further differentiated form of follicular

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stromal cells and several transitional forms were observed. A striking feature of dendritic reticular cells was that immune complexes were captured and retained extracellularly on their processes. The dendritic reticular cells were later termed follicular dendritic cells (FDCs) [3], a term that has helped to distinguish them from the phagocytic, interdigitating dendritic cells (DCs) in T cell areas. Some of the reticular cells present in primary follicles were also found to be immune-complex-trapping [4], and these cells are also now known as FDCs (Figure 1).

Several decades ago, it was established that deposition of immune complexes on FDCs was strongly dependent on complement [5–7] and the Fc portion of antibodies [7,8]. The complement receptors 1 (CD35) and -2 (CD21) were found to be highly expressed by FDCs [2]. In the mouse these complement receptors are alternative splice variants of a single locus [9]. Trapping of immune complexes on FDCs in GCs also occurs by a complement-independent mechanism that was found to be mediated by Fc γ RIIb [10,11].

2. Development of FDCs

FDCs are radiation resistant [12,13] and it has not yet been possible to definitively identify FDC precursor cells, making it difficult to study early FDC development. Most studies have focused on two major models for FDC development: 1) FDCs develop by further differentiation of a subset of follicular stromal cells of mesenchymal origin and 2) FDCs develop from migratory precursor cells. The evidence for each of these models will not be described in detail here, and the reader is referred to a previous review for further information [1]. Recent studies continue to support the conclusion that most FDCs are of mesenchymal origin, though not necessarily distinguishing whether they develop *in situ* or migrate to the follicle from another site. For example, the findings that FDC-like cell lines cultured from human tonsil express α -smooth muscle actin and exhibit some contractile activity were taken to favor the view that they are a specialized form of myofibroblast with similarities to bone marrow stromal cell progenitors [14]. Another recent study has suggested a novel mechanism of FDC development that involves both resident and migratory cells [15]. Specifically, the authors propose that an FDC is generated by a cell fusion event between a stromal cell and migratory CD35⁺B220⁺ precursor cell. This model is consistent with several observations of binucleate FDC [16,17]. A caveat to these studies is that the CD35⁺B220⁺ cells were only isolated to about 90% purity and had to be cultured for three days in order to obtain sufficient numbers of cells for experimentation.

Although the origin of FDCs remains incompletely defined, several requirements for FDC development have been established. An early finding was that B cells are required [18]. A large number of studies in the mid to late 1990s demonstrated that tumor necrosis factor (TNF) and the related molecule lymphotoxin (LT) are essential for FDC development, as mice deficient in these cytokines, their receptors, or associated downstream signaling molecules fail to properly develop FDCs and GCs in secondary lymphoid organs [1,19,20]. Through irradiation chimera and adoptive transfer experiments, it was established that TNF and LT were required on lymphocytes, whereas their receptors were required on radiation-resistant cells, for FDC development. Although studies of the relative requirements for TNF in B and T cells remain inconsistent [21–23], it has been clearly demonstrated that LT is required on B cells for normal FDC development [19].

TNF is expressed on the plasma membrane and can be shed to produce a soluble form. Mice expressing a mutant form of TNF that cannot be shed failed to form primary follicles and their associated FDC networks, but did form small GCs containing FDC networks after immunization [24]. These findings indicate that soluble TNF is essential for FDC development in primary follicles but that membrane-bound TNF may contribute to FDC development in GCs. In a recent study, TNF α converting enzyme (TACE, ADAM17), which is involved in the

shedding of several membrane-bound substrates including TNF and the TNF receptors, was shown to be critical in radiation-resistant cells for primary follicle development [25]. Although B cell numbers were diminished in these mice and further studies will be needed to identify the critical substrate(s) for TACE activity *in vivo*, it seems likely that the defective primary follicle development in these mice may be related to a requirement for soluble TNF in this process.

LT is a trimeric protein that can exist in two forms: a secreted homotrimer, LT α 3, or a membrane-bound heterotrimer, LT α 1 β 2. TNF and LT α 3 both bind the receptors TNFR1 (TNFRSF1a, TNFRp55) and TNFR2 (TNFRSF1b, TNFRp75) [20,26]. Studies in gene-targeted mice indicated a critical role for TNFR1, but not TNFR2, in FDC development [27–29]. A recent study has used a Cre-lox genetic method to show that expression of TNFR1 only in CD21/35-positive cells is sufficient for FDC development, suggesting that TNF and/or LT α 3 act directly on TNFR1 expressed by FDC precursors [30]. Whether or not soluble LT α 3 has a functional role *in vivo* remains controversial [26]. Membrane-bound LT α 1 β 2 binds a distinct receptor, the LT β R [20]. A profound defect in FDC development in LT β - and LT β R-deficient mice, as well as in mice treated with a blocking LT β R-human IgG1 fusion protein, suggest that membrane bound LT α 1 β 2 is the major contributor. The more severe phenotype originally reported in LT α 3-deficient mice may be due to diminished TNF expression caused by the insertion of a neomycin resistance cassette in the *Lta* locus; in a recently generated line of LT α -deficient mice in which the neomycin cassette was excised, the phenotype was similar to LT β -deficient mice [31]. A related ligand, LIGHT, can also bind the LT β R, although a role for LIGHT in FDC development has not been demonstrated [20,32].

Taken together, these studies have established critical requirements for TNF and LT in FDC development and have provided data on the cell types in which these molecules and their receptors have essential functions. However, a limitation to these studies is that many of them do not distinguish between FDC development in primary B cell follicles versus in GC.

3. Differences between FDCs in primary and secondary follicles

In primary follicles, FDCs are localized in the central region. In contrast, FDCs in GCs show a polarized distribution (Figure 1). FDC processes are most dense at the light zone pole of the GC, but some FDC processes can also be detected in the dark zone (Figure 2) [18].

Ultrastructural differences have also been described between FDCs in primary and secondary follicles, with the FDCs in GC light zones exhibiting a higher complexity of processes [2,33, 34]. FDCs in the light zone of GCs give a unique staining pattern with several monoclonal antibodies (Figures 1 and 2).

FDC in both primary and secondary follicles are recognized by antibodies to the complement receptors CD21 and CD35 [2]. As CD35 expression is low on follicular B cells, antibodies that specifically recognize CD35 give a fairly FDC-specific pattern of staining. CD35 antibodies stain FDCs in both dark and light zones of GCs (Figure 2). However, several other monoclonal antibodies to FDC antigens, such as FDC-M1 and FDC-M2, give much stronger staining on FDCs in GC light zones than in dark zones or in primary follicles ([35] and Figures 2 and 3). The FDC-M1 antigen is also abundant on tingible body macrophages in GCs (Figure 3) but its molecular identity remains undetermined. The FDC-M2 antigen was identified as complement component C4 in immune complexes, consistent with the finding that increased deposition of immune complexes and complement components is observed on FDCs in GC light zones compared with FDCs in GC dark zones or primary follicles [2,36]. As FDCs in both dark and light zones express complement receptors, other mechanisms must contribute to the selective trapping of immune complexes in GC light zones [2]. FDCs in GC light zones also upregulate two low affinity Fc receptors, CD23 (Fc ϵ RII) for IgE [37] [38] and Fc γ RIIb (CD32) for IgG

[11], although at least in the case of CD23 this only typically occurs in some tissues, such as in lymph nodes but not spleen [1], and under certain immunization conditions [38]. Recent *in vitro* studies have identified CD40, IL-4, and IFN- γ as potential inducers of CD23 on FDC [39]. While additional studies have suggested that FDCs express CD40 [2,40,41], by reconstituting irradiated CD40-deficient mice with wild-type bone marrow, we found that CD40 was not essential in FDCs for CD23 upregulation nor for grossly normal GC formation following immunization with nitrophenyl-conjugated chicken gamma globulin (NP-CGG) in complete Freund's adjuvant (C.D.C.A. and J.G.C., unpublished observations). FDCs in the light zones of GCs also upregulate several integrin ligands, including ICAM-1, VCAM-1, and MAdCAM-1 [2,42–44].

Taken together, the above findings indicate that light zone FDCs have acquired special properties, suggesting that they have further differentiated from FDCs in primary follicles. An alternative model is that additional cells recruited to GCs become part of the FDC network and express specific markers. LT and TNF are important for FDC development, as discussed above, and one possibility is that maturation from primary follicle FDCs to GC FDCs depends on increased signaling via LT and/or TNF receptors (Figure 1). Support for this model comes from the finding that GC B cells have elevated amounts of surface LT α 1 β 2 compared to naïve B cells [45]. *In vitro* addition of LT α 1 β 2 and TNF to FDC-like cells also led to the upregulation of several molecules associated with light zone FDC [46]. Quantitative differences in signaling via at least one other TNFR family member has been shown to lead to differences in cell fate [47,48]. However, it has not yet been shown whether elevated LT β R engagement is sufficient to promote maturation of primary follicle FDC to GC FDC. An alternative, non-mutually exclusive model is that additional inputs from activated lymphocytes or as result of other changes during the immune response are needed to promote FDC maturation.

Two recent studies have suggested that upregulation of ICAM-1 and VCAM-1 on FDCs in GC light zones can be induced by increased immune complex deposition in an Fc γ RIIb-dependent fashion [30,49]. In agreement with this notion, an earlier study found delayed light zone FDC maturation following SRBC immunization of Fc γ RIIb-deficient mice [50]. Whether Fc γ RIIb signaling induces FDC maturation directly or via actions in other cell types such as macrophages remains to be firmly established. Although Fc γ RIIb is best characterized for its SHIP-dependent inhibitory role in B cells, recent biochemical studies indicate the receptor can also engage a c-Abl dependent signaling pathway [51].

The finding that conditional deletion of IKK2 in FDC prevents ICAM-1 and VCAM-1 upregulation in GCs suggests that NF- κ B signaling in FDCs is involved [30]. Moreover, in cell preparations enriched for FDCs, NF- κ B was activated in an IKK2-dependent fashion in response to immune complexes, though possible contamination of the preparations by other cell types was not fully excluded. Another insight into the requirements for light zone FDC maturation comes from the finding that when B cells lack expression of CXCR4, FDCs with the characteristics of light zone FDCs appear throughout GCs in secondary follicles rather than being polarized toward the light zone [52]. Therefore, B cells contribute to the generation of light zone FDC at the correct pole by a CXCR4-dependent but as yet undefined mechanism (Figure 1).

Although the FDCs in GC light zones have been extensively described, the properties of FDCs in the dark zones of GCs remain unclear. Recently, the monoclonal antibody D46 was described that stained reticular cells in the dark zones of bovine and ovine GCs, and based on co-staining with antibodies to CD21 it was suggested that D46 specifically recognizes dark zone FDCs [53]. This antibody was found to recognize fibrinogen, which was suggested to deposit on FDCs in the GC dark zone, and other anti-fibrinogen antibodies also stained FDC in the GC dark zone in bovine mesenteric lymph nodes and human tonsils.

4. Functions of FDCs

4.1 Immune complex display

It is generally thought that a major *in vivo* role of FDCs is to present antigen in the form of immune complexes to both naïve B cells as they survey primary follicles and to GC B cells as they compete for antigen within GCs [13,18]. Immune complexes can be composed of antigen and antibody, antigen and complement, or all three molecule types. Complement containing immune complexes can often be formed very early after exposure to pathogens as a result of the alternative or lectin-pathways of complement activation or through binding by pre-existing cross-reactive ('natural') antibodies [9]. Immune complexes are rapidly transported and deposited on FDCs in primary follicles and GC light zones. Based on indirect evidence, early studies suggested that B cells may be involved in immune complex transport to FDCs [5,54], although other studies suggested a role for dendritic antigen transporting cells [55,56]. A recent study has directly demonstrated that immune complexes can be transported from the subcapsular sinus of lymph nodes to FDCs by naïve B cells [Phan et al., in press]. This antigen transport function required complement receptor expression by the B cells and it seems likely that the much higher complement receptor expression on FDCs than on B cells facilitates FDC capture of immune complexes from B cells. This study points out that the large immune complex trapping capacity of FDC networks may be important to ensure B cells are 'scrubbed' of any immune complexes they carry before returning to circulation. Thus, as well as facilitating local B cell responses, trapping of immune complexes by FDCs may be important to prevent systemic spread of any pathogens they contain.

Several recent studies have examined sites of B cell first encounter with antigen, revealing roles for antigen-display by DCs and subcapsular sinus macrophages as well as evidence of B cell encounter with soluble antigen within follicles [57,58][Phan et al., in press]. These studies have not yet examined the process of naïve B cell encounter with immune complexes displayed by FDCs in primary follicles and we can anticipate new advances in this area in the near future as real-time imaging approaches are applied to tackle this issue.

Within the GC, immune complex display by FDCs has long been suggested to provide a depot of antigen for which newly mutated B cells compete during the process of antibody affinity maturation [18]. A number of *in vitro* studies have demonstrated that FDCs can present immune complexes in a fashion that is highly stimulatory to B cells [59,60] and can induce expression of activation induced cytidine deaminase (AID), leading to somatic hypermutation and class switch recombination [61]. The highly conserved deposition of immune complexes on FDCs in all species that form GCs suggests that these immune complexes are likely to often be functionally important during the GC response. However, the importance of immune complex presentation by FDCs during the GC response *in vivo* has been difficult to test directly and is a subject of on-going debate, which has been discussed extensively in two opinion articles [59,62].

A focus of these articles was the finding that GCs of normal size formed and some evidence for selection was obtained in mice deficient in immune complexes [63]. While highlighting that immune complex deposition may not be essential for all steps of the GC response, this work does not exclude the possibility that immune complexes are normally involved in affinity maturation in GCs. Limited assessments of selection could be performed with this model and the reduced ability to form immune complexes may itself lead to a non-physiological elevation in free antigen due to impaired antigen clearance. More generally, it is plausible that localization of some antigens within GCs may require immune complexes, whereas other antigens may be distributed in soluble form. Indeed, upon immunization with a depot-forming adjuvant that might slowly release soluble antigen, CD21/35-deficient mice with defective

immune complex deposition were found to undergo normal GC and affinity maturation responses, whereas in the absence of adjuvant several defects were observed [64].

In addition to helping retain immune complexes within the GC light zone, FDCs are also likely to have qualitative effects on the way the antigens are displayed to B cells. Most prominently, this may be by displaying the complexes in a multivalent array [59]. Such immune complexes may be highly stimulatory to B cells by clustering their B cell receptors (BCRs) and CD21 molecules, yet conversely IgG in immune complexes can also be inhibitory by binding Fc γ RIIb [13,65]. It has been proposed that one mode of B selection in GCs is by competition between stimulatory BCR signals and inhibitory Fc γ RIIb signals following engagement of immune complexes, such that in higher affinity cells the relatively increased BCR signaling favors cell survival, whereas in lower affinity, and possibly autoreactive, cells, the relatively higher Fc γ RIIb signals favor apoptosis [65,66]. Another group has suggested that high expression of Fc γ RIIb by FDCs in GC light zones prevents interactions between the Fc regions of IgG in immune complexes with Fc γ RIIb on GC B cells [11], allowing immune complexes to be highly stimulatory to GC B cells.

Immune complex deposition on FDCs may also be involved in the differentiation of GC B cells into plasma cells or memory B cells. One model that has been proposed is that during the course of the immune response, as antigen-specific IgG is produced, engagement of IgG-containing immune complexes by Fc γ RIIb on GC B cells shifts differentiation toward memory B cells rather than plasma cells [67]. This model remains difficult to test directly, as Fc γ RIIb is also expressed in plasma cells and has been shown to have significant inhibitory action directly on these cells [68]. As immune complexes deposit rapidly on FDCs in secondary immune responses and FDCs can retain antigen for long periods of time, it seems likely that immune complexes deposited on FDCs are involved in memory cell maintenance and recall responses [13]. The need for persisting antigen in memory B cell maintenance remains controversial, however, due to particularities of the experimental systems used to test this idea [69,70].

Taken together, further studies are needed to establish the role of immune complexes deposited on FDCs in the initiation of B cell responses, GC function, and immunological memory, leading to long-term humoral immunity. In accord with the notion that immune complex deposition can be critical for long-term humoral immunity, in bone marrow chimera experiments where radiation-resistant cells lacked the expression of CD21/35, leading to a marked decrease in the trapping of immune complexes on FDCs, sustained IgG responses were diminished [71,72].

4.2 Chemokine expression and organizing functions

Work that has been previously reviewed provided evidence that CXCL13 (previously called BLC or BCA-1) is made broadly by follicular stromal cells, including the subset of these cells that correspond to primary follicle FDCs and GC light zone FDCs [1]. More recently, CXCL13 expression has been detected in FDC-enriched cell preparations [73], cultured FDC cell lines [14,74], and FDCs present within inflammation-induced ectopic lymphoid follicles [75–77]. CXCL13 and its receptor, CXCR5, are critical for B cell migration into follicles and are also required for normal migration of B cells to the light zone of the GC [1,52]. In some immunohistochemical analyses, CXCL13 was observed to be most abundant in association with the processes of follicular stromal cells and FDCs [1,52,78]. CXCL13 expression is strongly dependent on LT α 1 β 2 and to a lesser extent on TNF. CXCL13 itself directly induces LT α 1 β 2 expression by naïve B cells and this acts in a positive feedback loop to augment CXCL13 production [45]. Several studies have shown that CXCL13 can also be made by additional cell types such as macrophages, DCs, and follicular helper T cells [79–85] and it has sometimes been suggested that these cell types might be the more important follicular CXCL13 source. However, bone marrow chimera experiments established that the majority of

splenic CXCL13 expression is radiation resistant, supporting the earlier conclusion that the major source of follicular CXCL13 is follicular stromal cells [79]. Consistent with this conclusion, RAG-deficient mice that were reconstituted with CXCL13-deficient bone marrow were observed to form lymphoid follicles and to develop normal GCs in spleens and lymph nodes at the peak of the responses to immunization with sheep red blood cells or NP-CGG, respectively (C.D.C.A. and J.G.C., unpublished observations).

B cells migrate extensively within primary follicles and GCs and B cell motility is strongly dependent on *Gnai2* [86]. This movement is likely to be important to allow antigen-specific B cells to extensively survey the surfaces of antigen-presenting cells, whether FDCs, DCs or sinus-associated macrophages. The B cell movement has also recently been shown to facilitate delivery of immune complexes throughout the primary follicle and FDC network [Phan, 2007]. Two-photon microscopy studies indicate that the movement may at least in part be guided by FDCs [87–89]. Several studies have shown that CCL21 contributes to T cell motility within the T zone [90–92] and like CCL21, CXCL13 actively promotes chemokinesis *in vitro* [87, 93]. CXCL13 can be detected in amounts as high as 1ng/mg of total spleen and lymph node tissue (or 1µg/ml equivalent), indicating that local concentrations are likely to be in the range that is highly chemotactically active [94]. Two-photon microscopy analysis of GC B cell motility showed that it was reduced in the absence of CXCL13 [87] and it seems likely that this chemokine also promotes B cell motility in primary follicles. The high concentration of CXCL13 on FDC processes likely facilitates its role in promoting motility. Recent studies with CCL21 and SDF-1 (CXCL12) indicate that these chemokines are most active in promoting motility when presented in a surface bound form [Alon et al., in press].

In addition to the critical role of CXCL13 in organizing the lymphoid follicle and the GC light zone, SDF-1 plays a crucial role in organizing GCs into dark and light zones [52]. Positioning of GC B cells in the dark zone of the GC depends on their expression of the SDF-1 receptor, CXCR4 [52]. Immunohistochemical staining and laser capture microscopy demonstrated that SDF-1 mRNA and protein is more abundant in the GC dark zone than in the light zone while not being made by the GC B cells themselves [52]. These experiments implicate SDF-1 production as an important functional role for the sparsely distributed dark zone FDC network.

4.3 Adhesion molecules

The integrin ligands ICAM-1, MAdCAM-1, and VCAM-1 are abundant on the processes of FDCs in the GC light zone [42]. *In vitro* studies have shown that VCAM-1 can promote B cell attachment to GCs in a cryostat section [95], and that ICAM-1 and VCAM-1 can promote attachment of GC B cells to isolated FDCs [96]. VCAM-1 was also shown to promote adhesion of isolated T cells to an FDC-like cell line [97]. *In vitro* culture studies also suggest that ICAM-1 and VCAM-1 can prevent GC B cell apoptosis [98]. VCAM-1 on antigen-bearing membranes has been shown to facilitate B cell adhesion and activation [99]. It is notable, however, that no *in vivo* studies have been performed to directly test the role of these adhesion molecules or the integrins that they bind in GCs. Recent observations by two-photon microscopy that most B cells in GC light zones are motile suggest that these integrin ligands may function in a distinct manner *in vivo* from promoting firm adhesion of B cells to FDC processes [87,88,100].

Several studies that characterized the distribution of the polio virus receptor (CD155), an immunoglobulin superfamily molecule within the five member nectin subfamily of adhesion molecules, identified staining on tonsil and Peyer's patch FDCs [101–103] most likely corresponding to GC light zone FDCs. CD155 can interact with vitronectin and possibly also with nectin-3 [103]. Although the GC is relatively devoid of extracellular matrix components such as collagens, laminin and fibronectin [104], it contains vitronectin [2,101,105,106]. One possible activity of vitronectin is to inhibit the formation of the complement membrane attack complex, a role that may be important to protect GC B cells from complement cascade activity

that is so prominent within this microenvironment. Another possible function of vitronectin is to act as an additional integrin ligand within the GC.

4.4 B cell trophic factors

Primary follicles and GCs with their resident FDCs are thought to provide supportive niches for naïve B cells and GC B cells, respectively. Radiation-resistant stromal cells have been identified as a major source of the B cell trophic factor BAFF, needed for maintaining B cell homeostasis, while hematopoietic cells were identified as a more minor source [107]. The extent to which the radiation-resistant, BAFF-producing cells correspond to follicular stromal cells and more specifically, to FDCs, remains unclear. The spleens of mice that lack TNF, LT α LT β or B cells – and thus conventional FDCs – continue to express substantial amounts of BAFF transcripts [108]. Moreover, mice deficient in TNF, LT α , and LT β have not been reported to have marked reductions in B cell numbers [109]. These observations do not exclude that FDCs produce BAFF but they suggest that they are unlikely to be the sole stromal source of BAFF. Evidence that FDCs do express BAFF comes from the finding that FDC cell lines derived from human tonsils or mouse lymph nodes produced detectable BAFF [14,74] and FDCs freshly isolated from human tonsils expressed BAFF mRNA [110]. In immunohistochemical analysis, human tonsil was argued to show BAFF staining in GCs [111] whereas another study found prominent staining in interfollicular regions but little in GCs [112]. In mice deficient in BAFF or BAFF receptor function, the early phases of GC responses can occur but the GCs are found to decay prematurely, suggesting that BAFF has a role in sustaining the GC response [113,114]. However, even though recirculating mouse and human B cells have high amounts of receptor-bound BAFF [108,115], a recent study found that GC B cells do not [115], raising the possibility that BAFF availability in the GC is low. Further work is needed to determine whether BAFF is down-regulated in GC FDCs compared to primary follicle FDCs, and to what extent selectively ablating BAFF production in FDCs affects B cell survival and the GC response.

Another FDC-derived molecule that might have trophic functions has been identified in human tonsil FDCs and in an FDC-like cell line, HK cells, by the monoclonal antibody 8D6 [116]. *In vitro* co-cultures of tonsillar B cells or lymphoma cell lines with HK cells treated with 8D6 antibody or COS cell transfectants showed that this molecule, termed FDC signaling molecule 8D6, can promote B cell proliferation and plasma cell differentiation [116,117]. In a recently described HK-cell dependent mouse lymphoma model, treatment with 8D6 antibody blocked lymphoma cell proliferation [16]. The extracellular region of the 8D6 protein contains two domains homologous to the type A cysteine-rich repeats found in the low-density lipoprotein receptor family and complement components C8 and C9 [116], but the basis for its functional effects is not yet defined.

4.5 Cytokines

FDCs have been suggested to express an array of cytokines and cytokine receptors, based on immunohistochemistry of lymphoid tissues, analysis of FDCs isolated from these tissues, and analysis of FDC-like cell lines [2,118,119]. Each of these types of analyses has important limitations, however, and in most cases data for *in vivo* functional roles are lacking. In both immunohistochemistry and analysis of isolated FDCs, it also cannot be excluded that signal originated from nearby or contaminating cells. By contrast, FDC-like cell lines may be grown in pure form, but may also have undergone changes *in vitro* that do not reflect the phenotype of FDCs *in vivo*. Background staining on FDCs can be problematic due to their high expression of Fc receptors and captured antigen-antibody complexes.

For some cytokines and cytokine receptors suggested to be expressed by FDCs, possible *in vivo* functions have been described. As noted above, IL-4 may be able to alter CD23 expression

on FDCs and consistent with possible direct effects of this cytokine, the IL-4 receptor was present in an FDC-like pattern in GCs [118]. Mice lacking IL-6 mount GC responses of reduced magnitude [120,121] though it remains to be determined to what extent this reflects a paracrine requirement for FDC-derived IL-6. FDCs in some GCs have been reported to produce IL-6 based on *in situ* hybridization [122], immunohistochemical staining [120], and analysis of FDC cell lines [46,74,122], yet other studies have concluded that isolated FDCs do not produce IL-6 [17,123,124].

4.6 Additional FDC molecules of likely functional importance

Hepatocyte growth factor (HGF) and its activator were found expressed by FDCs in the GC dark zones of human tonsils, and B cells in the dark zone were reported to express the receptor c-Met [73]. Many possible functions have been described for HGF and further studies will be needed to determine the *in vivo* role of HGF in GCs. It has also been recently demonstrated that GC FDCs in human tonsils express Sonic hedgehog (Shh), and that tonsillar GC B cells express two components of the hedgehog receptor [125]. *In vitro* blockade of Shh promoted GC B cell apoptosis and addition of Shh to cultures reduced Fas-mediated apoptosis. FDCs have also been reported to express prostacyclin (PGI₂) synthase in human tonsils, and prostacyclin was shown to regulate the proliferation of T helper cells *in vitro* [126]. Recent *in vitro* studies of HK cells suggest that cyclooxygenase-2 may be more critical than prostacyclin synthase for the generation of prostacyclin by FDCs [127].

Another recent study focused on identifying FDC specific genes by comparing gene expression between mesenteric lymph nodes of control mice and mice treated with LT β R-human IgG1 to deplete FDCs, and by subtractive hybridization of FDC enriched versus depleted samples [128]. Although both techniques have technical limitations, some of the genes identified as differentially expressed by both approaches seem likely to be FDC-expressed genes. This study together with earlier work identified cellular prion protein (PrPc) as being expressed by FDCs [128,129]. The presence of PrPc on FDCs may contribute to prion replication [129] but this is controversial [130]. PrPc is also expressed on lymphocytes and various other immune cell types but its function in the immune system remains enigmatic [131]. Also identified in the expression analysis was the secreted glycoprotein, clusterin (apolipoprotein J), and staining analysis has confirmed that it is present within the FDC network [128,132]. The function of clusterin is poorly defined but *in vitro* experiments suggested it may be able to augment GC B cell survival [128]. Studies in other systems suggest that clusterin can be induced in viable cells that are exposed to dying cells and that clusterin then binds and facilitates clearance of apoptotic cell debris [133], a function that could be highly relevant in the GC.

FDC secreted protein (FDC-SP) is a proline-rich secreted peptide identified in human tonsil FDCs and in an FDC cell line [134]. However, the proline-rich nature of this peptide is typical of salivary proteins and comparative expression analysis indicates that it is most abundantly expressed in salivary glands ([135] and L.R. Shiow, Y. Xu, and J.G.C. unpublished observations) and no evidence has yet been obtained to demonstrate its expression by FDCs at sites other than human tonsil. A recent overexpression study in mice provided evidence that high systemic FDC-SP expression can diminish the magnitude of the GC response [136], though additional approaches such as gene knockout studies will be needed to determine whether this peptide has a physiological role in the GC.

The Fc α / μ receptor, a receptor with low affinity for IgM Fc regions and higher affinity for IgA Fc regions, has been found to stain GCs though the extent to which this corresponds to FDC versus B cell or myeloid cell expression remains to be established [137]. However, this observation does suggest that there may be further modes of immune complex capture within the GC microenvironment in addition to complement receptors, Fc γ RIIb, and CD23-mediated capture.

5. Concluding remarks

FDCs remain a challenging cell type to study due to the difficulty of isolating the cells in pure form and the lack of well developed genetic tools for selectively manipulating their gene expression. Despite these challenges, as a result of the increasing awareness of the likely importance of FDCs as support cells for the B cell response, a growing number of studies are examining their phenotype and function, especially within GCs, leading to a variety of new insights. In this review we have attempted to highlight the currently understood relationships among follicular stromal cells, primary follicle FDCs and GC FDCs, with the goal of providing a framework for considering the unique developmental requirements and functions of each cell subset.

A long-standing hypothesis has been that the major function of primary follicle and GC FDCs is to display immune complexes. Recent findings have highlighted other ways in which B cell encounter with antigen can occur [57,58][Phan et al., in press] and they continue to leave as an open question the extent to which FDCs are required for this process. The findings that we have reviewed above suggest that FDCs express a number of molecules that have important functions in B cell biology, and it seems likely that these molecules may be as critical as immune complex capture for FDC interactions with B cells in primary follicles and GCs.

An area where insight is only beginning to be obtained is the extent to which FDCs can respond to cytokines and innate immune stimuli, perhaps adjusting their function to favor different outcomes in different follicular and GC responses. One study found that during acute sepsis there was rapid and transient upregulation of FDC-M1 staining throughout the follicular region of the spleen, perhaps indicating direct stimulation of FDC maturation by TLR ligands [138]. IL-4 and IFN- γ have also been shown to have effects on FDC in culture [39,46] and it seems possible that these and other cytokines will act on FDCs (directly or indirectly) to modify their functions in polarized immune responses. The GCs that form in mucosal lymphoid tissues may have different properties from those that form at other sites and these mucosal GCs might require additional specializations in FDC function [139,140]. Finally, it remains unclear how FDCs adjust as the GC response subsides and whether the cells undergo apoptosis or de-differentiate to primary follicle FDC. It is also unclear to what extent changes in the FDCs control the GC contraction phase. Certainly the ability of FDCs to harbor intact antigens on their surface for months and possibly years suggests that some FDCs survive well beyond the contraction phase of the GC response [141].

As the understanding of FDC biology advances there is certain to be an increasing number of clinical implications that emerge. TNF antagonists are in widespread clinical use for treatment of rheumatoid arthritis and several other diseases. These treatments might be anticipated to reduce FDC function and thus humoral immunity, though to what extent is not yet clear. The significance of FDCs as a supportive niche is likely to extend to B cell responses at sites of autoimmune inflammation [75–77,142] and may also be the case for various types of B cell lymphomas [40]. It will be important to assess the effects on these diseases of disrupting FDC function, as might be achieved, for example, by treatment with LT and TNF blocking agents [143] or antibodies against some of the newly described FDC surface molecules. Because of their ability to trap and retain immune complexes intact for long periods, FDCs are thought to be an important reservoir of viruses such as HIV [144]. Studies of the requirements for HIV virion deposition on FDCs in model systems have been initiated [145,146] and may lead to new approaches to deplete pathogens from these potentially dangerous depots.

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Figure 1. Schematic representation of follicular stromal and FDC networks in a primary follicle and in the same area after maturation into a GC-containing secondary follicle

Follicular stromal cells (FSCs) and FDCs are suggested to form a contiguous network. Primary follicle FDC are suggested to undergo maturation into light zone (LZ) and dark zone (DZ) FDC in response to signals that include LT, TNF and immune complexes (IC) as well as still unknown (other) signals. Surface markers that typify the cells are listed along with an indication of their relative abundance. The absence of a marker (–) indicates below the level of detection by conventional immuno-staining procedures. Variable reports in the literature indicated as +/–. *, special note as follows: FcγRIIb^{+/-} indicates functional evidence only; CD23 is present on LZ FDC in only certain GCs; IC-trapping in DZ demonstrated only after *in vitro* incubation; Fibrinogen associated but not expressed.

Figure 2. FDC marker distribution in GC light and dark zones

Serial 7 μm cryostat sections of a draining peripheral lymph node from a mouse that had been immunized s.c. 14 days earlier with NP-CGG in complete Freund's adjuvant are shown. Sections were stained with antibodies to the indicated markers. GCs are identified as areas deficient in IgD⁺ B cells and positive for GL7 antibody staining. Note the presence of CD35⁺ FDCs in the dark zone that are negative for the light zone FDC markers CD23, FDC-M2 and FcγRIIb. VCAM-1 staining is observed on tingible body macrophages in addition to FDCs. Scale bar, 100 μm.

Figure 3. FDC-M1 staining of GC light zone FDC and tingible body macrophages (TBM)
Tissue preparation and analysis was as in Figure 2. Scale bar, 50 μ m.