Extrafollicular antibody responses

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Summary: In adaptive antibody responses, B cells are induced to grow either in follicles where they form germinal centers or in extrafollicular foci as plasmablasts. Extrafollicular growth typically occurs in the medullary cords of lymph nodes and in foci in the red pulp of the spleen. It is not a feature of secondary lymphoid tissue associated with the internal epithelia of the body. All types of naïve and memory B cells can be recruited into extrafollicular responses. These responses are associated with immunoglobulin class switching but, at the most, only low-level hypermutation.

Introduction

Extrafollicular responses are responsible for the fast production of antibodies after antigen encounter and can be divided into four phases; each of these phases occurs in a different microenvironment (*Figs. 1 and 2*). The response starts when B cells bind antigen through their surface immunoglobulin (Ig). This binding induces the B cells to migrate to the T-cell-rich zones of secondary lymphoid organs, where they efficiently interact with antigen-primed T cells. Antigen encounter in the blood or marginal zone induces migration to the splenic T zone. Lymph node B cells binding antigen in afferent lymph move to interact with primed T cells in the same node. When a primed T cell recognizes antigen presented by a B cell, local growth of both is induced. After two cell cycles, B blasts that have been induced to become plasmablasts migrate from the T zone to the local site of extrafollicular growth. During this growth and the subsequent differentiation to plasma cells, there is no contact with T cells. Nevertheless, association with $CD11c^{high}$ dendritic cells appears to be required for full differentiation of plasmablasts to plasma cells. A proportion of the plasma cells produced in extrafollicular responses gain access to stroma that secures their extended survival. Certain antigens induce extrafollicular antibody responses without T-cell help. In these responses, B cells pass through the same sequence of microenvironments.

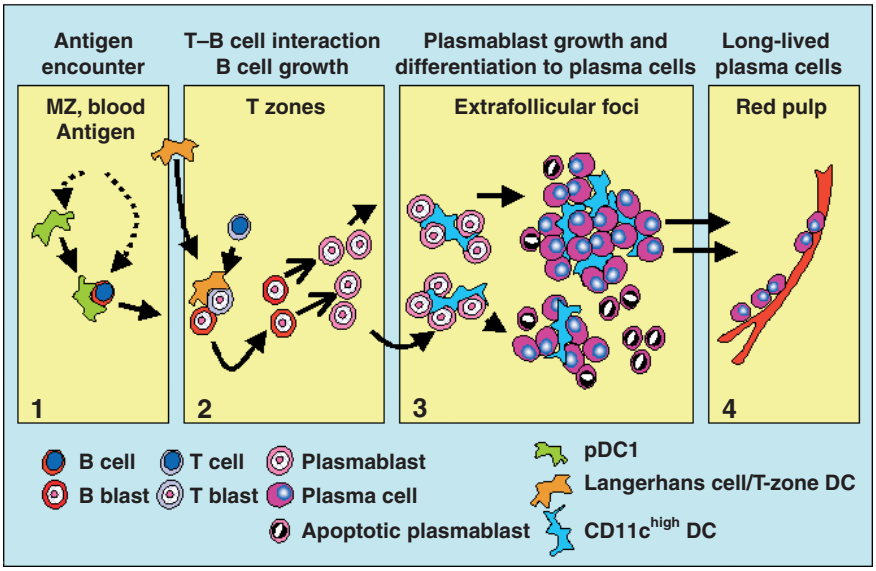

Fig. 1. The four phases of splenic extrafollicular antibody responses occur in distinct microenvironments. MZ, marginal zone, DC, dendritic cell, PDCI, immature DC with capacity to transport intact antigen.

Three pathways to antibody production

The maturation of B cells to plasma cells can occur in three main ways. First, B1 cells can mature with or without external antigenic stimulus to become IgM or IgA producers. It has been estimated that up to half the IgA secreted from the gut in mice is produced in this way (1). Normal serum IgM antibody concentrations are reached in germ-free mice fed on a chemically defined diet and maintained in a hypoallergenic environment (2). Although the repertoire of this 'natural' antibody is far more restricted than that produced in conventional mice (3), it is likely to have an important protective role in infancy. The second route to antibody production is when B cells that have engaged antigen are induced to grow, with or without CD4 T-cell help, as plasmablasts in extrafollicular foci in the spleen or in the medullary cords of lymph nodes. This pathway is the subject of the present review. Finally, plasma cells are generated from germinal center B cells in the course of follicular responses (4, 5). These cells originate from a proportion of the B cells that bind antigen and then make a cognate interaction with primed CD4 T cells (6, 7). B cells growing in follicles activate a site-directed hypermutation

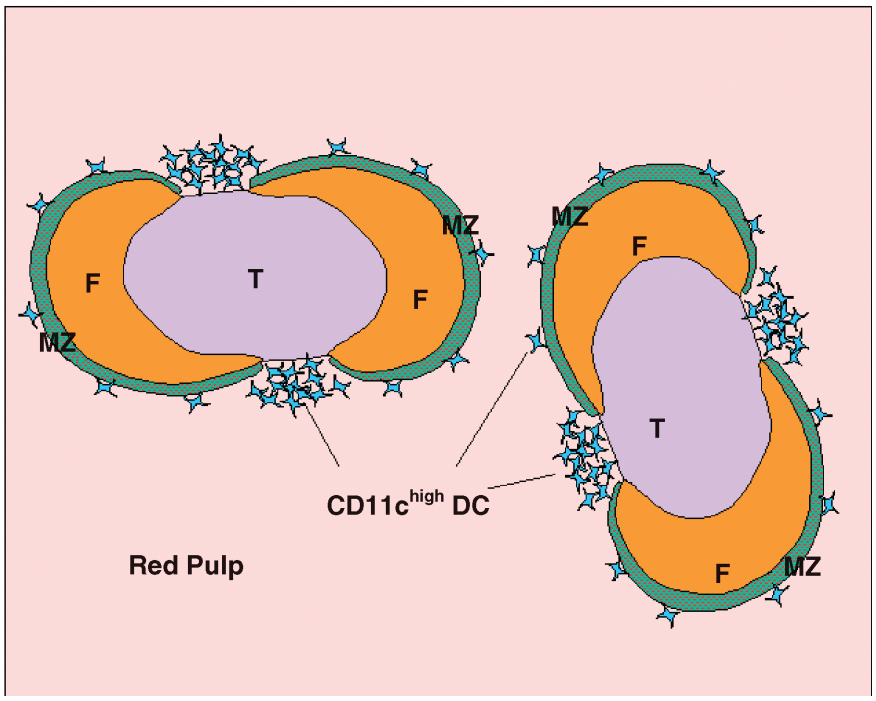

Fig. 2. The main splenic compartments and their relation to CD11c^{high} plasmablast-associated dendritic cell. The red pulp and marginal zone are perfused by an open sinusoidal blood supply. T, T zone; F, follicle; MZ, marginal zone.

mechanism, which introduces mutations in the rearranged immunoglobulin variable region genes (8). Periodically, germinal center B cells are subjected to antigen and CD4 T-cell-mediated selection (9, 10). A proportion of the selected cells leave the germinal centers and differentiate into plasma cells, which colonize the bone marrow (4, 5) or tissues underlying the internal epithelial surfaces (11).

Phase1: B-cell engagement with antigen

A major proportion of mature naïve B cells are recirculating cells (12). They do not visit the main sites where antigen enters the body, i.e. the epithelial surfaces. Most of these cells are located in the follicles of secondary lymphoid tissues, but they only stay in a follicle of one lymphoid organ for around a day before migrating to the blood either directly or via efferent lymph (13). They then enter another secondary lymphoid tissue by binding to molecules expressed on the surface of specialized vascular endothelium. They first enter the T zone and then migrate to the follicles where the cycle begins again. If these B cells encounter antigen in the blood, they will selectively migrate into the T zone of the spleen (7, 14). In lymph nodes, recirculating B cells move along the walls of intranodal lymphatics on their way to the follicles (15, 16). Theoretically, while in this location, they can contact free antigen in the lymph. In addition, there is evidence that a subset of dendritic cells (DC) can transport antigen to recirculating B cells in lymph nodes (17). These recirculating B cells clearly get access to antigen efficiently, for they are rapidly recruited into both follicular and extrafollicular T-cell-dependent antibody responses in lymph nodes (18, 19).

A second subset of B cells is a nonrecirculating population that comprises a mixture of naïve (20–22) and memory cells (23). These collectively are termed marginal zone B cells, for they were first identified in the marginal zone of the spleen (24). Their phenotype is different from that of recirculating cells. They are larger, have less condensed chromatin and express higher levels of costimulatory molecules (CD80 and CD86) than recirculating follicular B cells (25). Marginal zone B cells express higher levels of IgM and less IgD (12, 26) as well as high levels of CD21 expression and low levels of CD23 (27, 28). These phenotypic differences reflect different activation requirements of recirculating and marginal zone B cells. Most significantly, marginal zone B cells, including memory B cells but not recirculating B cells, respond to thymus-independent type 2 (TI-2) antigens *in vivo* (22, 28, 29). Cells with phenotype similar to marginal zone B cells are found in patches along the inner surface of the subcapsular sinus of lymph nodes, particularly the mesenteric lymph nodes (26), in the crypt epithelium of the tonsil (30) and under the dome epithelium that covers the surface of Peyer's patches (31). Marginal zone B cells do not recirculate between blood and lymph, although memory B cells are found in the blood (32). Those marginal zone B cells found in lymphoid tissues remain in their compartment (12) unless they are activated by antigen, which induces them to translocate rapidly to the local T zone (7, 23). The location of marginal zone cells is such that they are well placed to contact antigen. Thus, many but not all splenic marginal zone cells are perfused by blood sinusoids. The inner layer of the marginal zone in humans has a capillary blood supply. Immature DC in the blood have recently been shown to transport intact bacterial antigen to marginal zone cells (33). The tonsil marginal zone cells are likely to be able to bind antigens entering the tonsilar crypts, and those marginal zone cells in lymph nodes are located in the inner wall of the subcapsular sinus and can capture antigens from lymph.

Phase 2: induction of B-cell proliferation in the T zone

The frequency of naïve T and B cells specific for antigens associated with many pathogens is in the order of one in many thousands. Consequently, the chance of a naïve B cell and a naïve T cell that recognize the same antigen being adjacent to one another is remote. The odds are shortened to a virtual certainty by the following changes. Firstly, B cells acquire the capacity to interact with T cells after they have specifically engaged antigen, and secondly, T cells that have been primed by antigen provide signals that attract the B cells that have taken up antigen. Antigen localization means that these two antigen-primed partners will be located in the T zones of the same secondary lymphoid tissues. This section considers the events that lead to this cognate interaction between T and B cells and its immediate consequences before the T and B cells move apart.

Interaction between primed T cells and B cells that have taken up antigen

Antigen-induced migration of B cells from the marginal zone compartments or blood to the T zones was described in the previous section. Recirculating B cells (13) and immature B cells (34, 35) enter the outer T zones of secondary lymphoid tissue without binding antigen. The recirculating B cells pass on to the follicles, and the immature B cells will die within a day or two, unless they receive signals that recruit them into one of the mature B-cell pools (36). Neither are programmed

to interact with primed CD4 T cells in the T zone, but the recirculating (37) and immature (38) B cells on binding antigen rapidly acquire this capacity. This progression can be shown in mice with CD4 T cells that have been primed against a protein antigen. If the primed mouse is challenged several weeks later with soluble hapten–protein conjugate, the speed of recruiting naïve hapten-specific B cells can be compared with the rate of activation of memory B cells specific for the carrier protein. Both grow with the same kinetics and come out of cell cycle as plasma cells at the same time (39). Within 12 h of challenge, specific B cells have taken up and processed the antigen, presented it on their surface and migrated to interact with carrier-primed CD4 T cells. In addition, during this first 12 h, they receive signals from the T cells and in response produce Ig heavy chain switch transcripts (14). Migration of B cells that have bound antigen to the T zone may in part be a consequence of their increased expression of CCR7, which ligates the T-zone chemokines CCL19 and CCL21 (40). In studies of human tonsil B cells, activation through their B-cell antigen receptor (BCR) increased migration to CCL19, but this movement was not associated with a major upregulation of CCR7 (41). This and other molecular changes that allow previously naïve B cells to find primed T cells are described by Dr Cyster in his review in this volume.

In response to certain antigens, specific CD4 T cells can acquire the capacity to attract and interact with B cells that have taken up antigen within 24 h of primary immunization. This has been found in responses of mice to hapten–protein conjugates given with bacterial cell wall lipopolysaccharide (LPS), which is a strong innate inducer of antigen processing by tissue DC (42). Priming may be slower if less powerful innate signals are available to activate the tissue DC at the site of immunization (18, 19). Cognate T-cell interactions with B cells start around the time the T cells enter the cell cycle (43). The B cells are induced to produce switch transcripts in G1 before they first enter S phase. Thus, the lag between a naïve CD4 T cell making cognate interaction with a DC and a B cell that has taken up antigen joining this conjugate is well under a day. The main time limiting factor is likely to be the time it takes for DC to be activated at the site of infection and migrate to the T zone of adjacent secondary lymphoid tissue.

Induction of switching during primary B-cell activation

The isotype of the Ig heavy chain produced by a B cell is related to the antigen inducing the response. As a necessary prelude to Ig class switching, heavy chain germline transcripts (switch transcripts) are produced (44). Their production is necessary for switching, but switch transcript production by itself is not necessarily followed by switch recombination (45). Examples of antigen-related switching include responses to alum-precipitated protein, where γ1 and ε switch transcripts are produced, while γ2a switch transcripts are generated in response to killed *Bordetella pertussis* (19, 43). This divergent switching, respectively, reflects T helper2 (Th2) and Th1 activity. *In vitro*, switching to IgG1 and IgE is induced in LPS or CD40-stimulated B cells cultured in the presence of interleukin-4 (IL-4) (46, 47). Conversely, when γ-interferon is substituted for IL-4, the switching is to γ2a (48). It is tempting, therefore, to deduce that the directional switching induced early in T-dependent (TD) extrafollicular antibody responses is brought about by these cytokines. Some doubt about this conclusion was raised when we analyzed responses to mixtures of alum-precipitate hapten–protein and killed *B. pertussis*. As indicated above, when given alone these, respectively, induce Th2 and Th1 patterns of switching. Surprisingly, when these antigens are mixed, switching in the hapten-specific response remains almost exclusively to IgG1, despite the production of IgG2a-producing plasma cells in response to the *B. pertussis* (19). This finding excluded the direction of switching by ambient cytokines in the node, but it did not exclude the possibility that cytokines might be acting across the synapse between T and B cells created during cognate interaction. Additional experiments show that switching to IgG1 in B cells recruited into an extrafollicular response to the same antigenic mixture is unimpaired in mice doubly deficient in IL-4 and IL-13 (43). Thus, type 2 cytokine-directed switching seen *in vitro* does not appear to reflect the mechanism inducing switching to IgG1 in extrafollicular TD responses *in vivo*.

Commitment to the follicular or extrafollicular pathway of B-cell growth

This divergence of B-cell differentiation leads on the one hand to germinal center formation and on the other to extrafollicular growth as plasmablasts. Although much is understood of the changes in phenotype associated with cells embarking on both these pathways, very little is known of the signals that induce cells to differentiate in one direction or the other. When a B cell moves along the follicular route, it upregulates the transcriptional repressor Bcl-6, and this factor inhibits differentiation towards plasmablasts by repressing B lymphocyte-induced maturation protein-1 (Blimp-1), the key transcriptional repressor required for plasma cell differentiation (49). Indeed, enforced expression of Bcl-6 inhibits plasma cell

differentiation. Conversely, plasmablast differentiation is associated with the upregulation of Blimp-1 expression (50, 51). The targets of Blimp-1 suppression have been elucidated recently (51). Blimp-1 directly suppresses PAX-5, which is a dual function activator and suppressor and is required for germinal center formation. Genes activated by PAX-5, include molecules in the BCR signaling pathway such as CD19, CD79α, syk, and BLNK, all expressed in germinal center B cells and downregulated in plasma cells. Importantly, PAX-5 suppresses transcription of X-box binding protein-1 (XBP-1) (52), which was shown to be essential for plasma cell differentiation, as well as transcription of J chain, IgH and possibly Igκ. Regulation of XBP-1 activation does not only occur at the level of transcription, but it can also be spliced into a more active form by endoplasmic reticulum (ER) transmembrane proteins upon activation of the unfolded protein response pathway (UPR) (53). The UPR pathway regulates the protein folding capacity of the ER and can influence cellular metabolism and survival. Although this pathway is generally known to be initiated as a stress response, in antigen-stimulated B cells it is activated prior to IgG secretion and might play a critical role not only in handling the proper folding and assembly of large amounts of immunoglobulin chains in the ER but also at an earlier stage regulating B-cell differentiation (54).

The commitment to one lineage or the other probably occurs at an early stage. Blimp-1 is already upregulated within 18 h of immunizing with the TI-2 antigen NP-Ficoll (55). Classically, germinal centers are T-cell dependent (56) and are not associated with TI-2 antibody responses. This relationship is true for productive germinal centers, but it has become clear recently that TI-2 antigens can induce abortive germinal center development (57, 58). This development includes the upregulation of Bcl-6 and many of the genes expressed in developing germinal centers (59); the nascent germinal centers undergo involution after 4 days, without producing plasma cells or memory (57). Their demise is probably associated with failure of the germinal center B-cell selection process, which starts on the fourth day after primary B-cell activation (60). It is possible that the induction of germinal centers without extrafollicular plasmablast growth occurs in the lymphoid tissue associated with epithelia, but the possibility that some B cells activated in these tissues grow as plasmablasts in the medullary cords of downstream lymph nodes remains to be explored. There is a relative but not an absolute bias of mice deficient in Btk to produce extrafollicular rather than follicular antibody responses (61).

Studies by Jacob and Kelsoe (62) show ipsiclonal B cells in extrafollicular foci in the spleen and adjacent germinal centers. This finding might indicate that B cells complete at least one cell cycle before committing to either follicular or extrafollicular differentiation. There are two reasons for querying this conclusion. First, emigrant plasma cells from germinal centers pass through the area of the spleen where extrafollicular plasmablast growth occurs. They have been found in that site as early as five days after immunization and can become long-lived plasma cells in the splenic red pulp (39). Second, the clonality and growth kinetics of germinal centers indicates that three B cells on average give rise to some $10–15 \times 10^4$ germinal center cells in 96 h (7). This implies an average cell cycle time of around 6 h. As will be discussed below, the first two cell cycles in an extrafollicular response occur more slowly (55).

There is some evidence to suggest that marginal zone B cells are preferentially and more rapidly recruited into extrafollicular antibody responses than recirculating B cells, while recirculating cells can be recruited to both follicular and extrafollicular antibody responses. Separate B cell subsets contributing to these responses was first suggested by Klinman and Linton (63). Antibody produced in responses to TI-2 antigens results from extrafollicular growth of marginal zone B cells, and recirculating B cells do not appear to be recruited into these responses *in vivo* (22, 28, 29, 64). Conversely, the dual potential of recirculating cells is suggested by the brisk follicular and extrafollicular response to TD antigens induced in popliteal lymph nodes from young specific pathogen free mice. These nodes, unlike mesenteric nodes have very few, if any, marginal zone B cells. It is unclear if marginal zone cells can be recruited into follicular responses. This possibility requires further analysis. Taken together, these data indicate that while certain antigens may be more likely to recruit one subset of B cells to an extrafollicular response and another to follicular growth, there is no absolute restriction of either response to a single B-cell subset. The way signals delivered through the B-cell receptor and other B-cell surface molecules cause B cells to differentiate in different directions is only partially understood. Most of the transmembrane and intracellular signaling molecules involved may already be known. The difficulty is understanding how these signals are orchestrated to achieve distinct end results.

B-cell proliferation in the T zone

B cells recruited into extrafollicular responses enter into cell cycle in the T zone. We have studied the early phase of the splenic extrafollicular response to NP-Ficoll. Mice that carry an Ig heavy chain transgene (65) that endows some 10% of the

B cells with NP-specificity were used (55). The NP-specific B cells moved from the marginal zone to the T zone within 8 h (57), and Blimp-1 is upregulated by 18 h. This process probably occurs in G1 before the B cells enter their first antigen-induced S phase. The B cells remain in the T zone until around 48 h after immunization, when they already express the plasma cell marker syndecan-1 (CD138). They then start to colonize the red pulp around the outer surface of the marginal zone and in the area where the red pulp joins directly with the T zone. Studies of proliferation of transferred carboxyflourescein diacetate succinamidyl ester (CFSE)-labeled transgenic B cells show that they have completed two cell cycles by 48 h after immunization. Whether this rather slow early proliferative response applies to physiological antibody responses in wildtype mice has to be determined. For example, it is conceivable that in this model, TI-2 large numbers of antigen-specific B cells overwhelm the stroma that might be critical for B-cell growth and survival.

Phase 3: plasmablast growth and terminal differentiation

The move from the T zone to the red pulp seems to represent a distinct checkpoint in the extrafollicular response, which is indicated by the response of B cells in Btk-deficient mice. The B cells of these mice go through the T zone phase of the response to NP-Ficoll, upregulating Blimp-1 and completing two cell cycles, but they die without entering the phase of growth in the red pulp (55). Once in the red pulp, association with CD11c^{high} DC (*Fig.* 2) appears to be necessary for continued plasmablast survival and differentiation to plasma cells (66). While the association of plasmablasts and CD11c^{high} DC appears critical, the translocation from the T zone to the red pulp is not strictly necessary for continued plasmablast growth. In T-cell-deficient mice, CD11c^{high} DC are located in the T zone rather than the red pulp, and although the anatomical shift of plasmablasts from the T zone to the red pulp does not occur, plasmablast survival and differentiation are normal (66). The CD11c^{high} DC and ectopic plasmablast growth are considered in more detail in the next section.

The plasmablast growth phase is associated with progressive downregulation of surface Ig, B220 (CD45), CD19, CD79α, and β, and other molecules associated with B-cell activation. Class II MHC molecules and the costimulatory molecule CD86 are also lost, reflecting a loss of capacity to make cognate interaction with T cells. Blimp-1, after transient downregulation at 24 h after immunization, rises to peak levels at 48 h. The transcription factor XBP-1, which is associated with and necessary for plasma cell differentiation, is upregulated (54) along with J chain and IgH chain expression. The activation-induced deaminase (AID) is a prime mediator in Ig class switch recombination (67, 68). AID is likely to be upregulated early in phase 3, for switch recombination programmed during cognate interaction at the start of phase 2 takes place during phase 3. Typically, Ig secretion and CD138 expression progressively increase during phase 3, which draws to a close as a proportion of the plasmablasts come out of cell cycle as fully differentiated plasma cells. Those cells not becoming plasma cells die as plasmablasts.

CD11c^{high} dendritic cells and factors associated with plasmablast growth, survival, and differentiation

We are beginning to understand the factors that result in some plasmablasts differentiating into plasma cells while others die. The first clue came from studies of a novel type of DC found in the medullary cords of lymph nodes, in the spleen in extrafollicular foci, and variably along the outer edge of the marginal zone. These DC characteristically express high levels of CD11c. Although many DC in the T zone express this molecule, which forms a heterodimer with CD18, the level of expression is substantially lower, as assessed by immunohistology (66, 69). The CD11c^{high} DC also differ from T zone CD11c^{+} DC by not attracting naïve CD4 T cells to their surface. In addition, neither plasmablasts in splenic extrafollicular foci (70) nor those in medullary cords of lymph nodes (18) attract antigen-specific CD4 T cells.

There is a strong correlation between plasmablast association with CD11c^{high} DC and their continued survival and differentiation into plasma cells (66). This link is seen particularly clearly in responses of mice with transgenic B-cell receptors. Thus, in QM mice with large numbers of NP-specific B cells, extrafollicular antibody responses against haptenated antigens result in the splenic red pulp being packed with plasma cells by four days after immunization with NP-Ficoll (39, 57, 66). From this situation there is mass apoptosis, so that by the sixth day of the response NP-specific antibody containing cells are confined to the proximity of CD11c^{high} dendritic cells in extrafollicular foci (66). The possibility that plasmablasts are attracted to CD11c^{high} DC is raised by finding that when these DC are located ectopically, plasmablasts are collocated with them. The example of their collocation in the T zone in T-cell-deficient mice has already been cited (66). Recently, we have reported a striking shift of both plasmablasts and CD11c^{high} dendritic cells from their physiological site during an extrafollicular response to murine malaria infection. In the first week after infection with *Plasmodium chabaudi chabaudi*, there is a strong extrafollicular antibody response with plasmablast

growth in the red pulp. As the parasitemic phase peaks at around day 10, the red pulp becomes depleted of leukocytes. At this stage, the plasmablasts and CD11c^{high} DC appear to translocate to the T zone, but in reality they form a distinct compartment well demarcated from CD4 T cells and conventional DC (71). While these findings suggest a mutual affinity of plasmablasts and CD11c^{high} DC, the development of the extrafollicular response in lymph nodes indicates that both are attracted to nascent medullary cords that contain neither cell type. This situation seems analogous to the one where the chemokine CXCL13 is produced in low amount by stromal cells in the nascent follicles of RAG-1-deficient mice to attract transferred recirculating B cells expressing the receptor for CXCL13 (CXCR5) to that site. Nevertheless, once the follicles are established and follicular dendritic cells are induced, these cells become the primary producers of CXCL13 (72). Available evidence suggests that CXCR4 expression by plasmablasts may be responsible for their homing to extrafollicular foci (73, 74). The chemokine CXCL12 (which is bound by CXCR4) is expressed in the splenic red pulp, medullary cords of lymph nodes and in the bone marrow (74). Plasmablasts deficient in CXCR4 fail to migrate to normal sites of antibody and CXCL12 production in the spleen. It is not clear if CD11c^{high} dendritic cells produce CXCL12 and whether other red pulp and medullary cord stromal elements produce this chemokine as well. Successful migration of plasmablasts to their site of extrafollicular growth is also probably influenced by the downregulation of CXCR5 and CCR7, which decreases their responsiveness to follicular and T-zone chemokines (CXCL13, CCL19, and CCL21) (74). It has been shown that transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) ligands play a key role in TI antibody responses and promote B-cell survival and activation at a number of levels (33, 75–77). They appear to act by promoting survival and secretion in plasmablasts, while they have little role in the induction of B cells to proliferate or differentiate into plasmablasts (33). It remains to be seen if CD11c^{high} DC are the source of the TACI ligands, B-cell-activating factor (BAFF or Blys) and/or a proliferation-inducing ligand (APRIL). These ligands, in addition, can promote Ig class switching *in vitro* (78), although it is not clear if they have a role in completing the class-specific switching reaction induced by T cells or the selective switching to IgG3 induced by TI-2 antigens (79). The switching induced by BAFF and APRIL *in vitro* is not isotype selective and in this respect mimics the effect of interferon (IFN)-α-induced switching *in vivo* (80).

As mentioned above, TACI-ligands do not appear to initiate or sustain proliferation of plasmablasts, other than by keeping them alive (33). Studies of plasmablast proliferation in extrafollicular responses found that the peak number of plasmablasts produced correlated directly with the number of B cells recruited into the response. Thus, when low numbers of B cells were recruited into a response, there was no compensatory increase in the number of cell cycles by the plasmablasts (39). This finding suggests that plasmablast growth, unlike the terminal differentiation of plasmablasts, is not limited by stromal capacity.

Despite these findings, it is clear that CD40 ligation *in vivo* can extend plasmablast growth in extrafollicular responses to TI-2 antigens by about 2 days (81). This response was achieved experimentally by administering agonistic antibody against CD40. More than one mechanism may contribute to this effect. Ligation of the CD40 on the B cells may in part be responsible, as suggested by the ability of CD40 ligation to overcome the block of Btk-deficient B cells in making the transition from T-zone blast to red pulp plasmablast (55). On the other hand, agonistic anti-CD40 antibodies cause a massive increase in the number of CD11c^{high} dendritic cells, which is achieved at least in part by driving proliferation of these DC *in situ*. The CD11c^{high} DC spread throughout the red pulp of the spleen and are associated with extended growth and increased final numbers of plasma cells (81). While the antibody titers are substantially augmented, the switching in the response remains strongly restricted to IgG3 production. Studies that have not previously been published show that agonistic CD40 antibodies, given at the site of subcutaneous immunization with alum-precipitated protein, massively increased the extrafollicular TD response and the proportion of cells switching to IgG1. Reflecting the effect in the spleen, the greatly enlarged medullary cords contained large numbers of CD11c^{high} dendritic cells and CD138^{+} cells (*Fig.* 3). As has been described by others (82), we find that treatment with these agonistic antibodies blocks both TI and TD germinal center formation, although the mechanisms remain to be determined.

Cell cycle control plays a key role in the differentiation of B blasts to antibody secreting cells. One of the targets of Blimp-1 transcriptional repression is c-Myc. Enforced expression of either c-Myc or cyclin E sustains proliferation and blocks cytokine-driven differentiation to Ig-secreting plasma cells in a mouse lymphoma cell line (83). Studies on IL6-induced differentiation of human B lymphoblastoid cell lines linked the expression of the CDK6-inhibitor p18^{INK4c} with cell cycle arrest and antibody secretion (84). The transition from plasmablast to plasma cell, which by definition involves coming out of the cell cycle, is associated with increased antibody

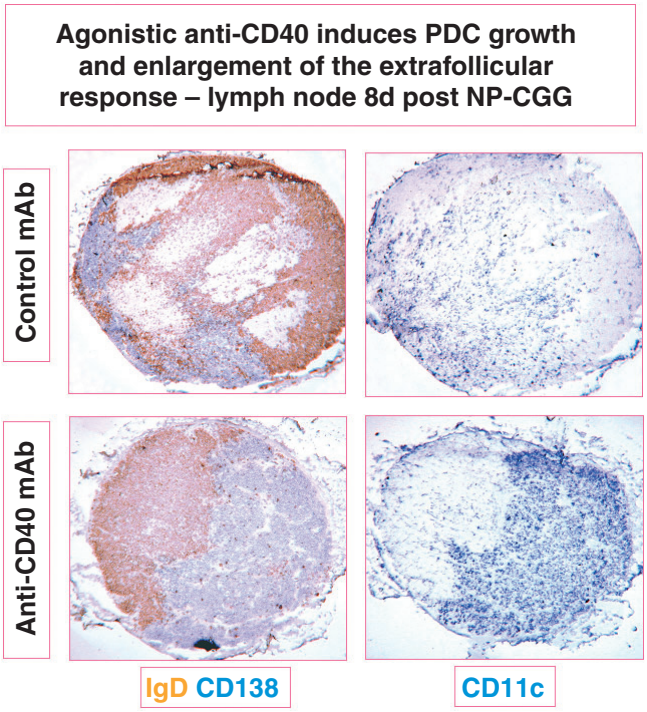

***Fig. 3.* The massive accumulation of CD11c^{high} plasmablast-associated dendritic cells (PDC) in the medullary cord of a popliteal lymph node induced by agonistic CD40 antibody given after immunizing mice with alum-precipitated hapten–protein conjugate (NP-CGG) in the foot.** The upper two panels show the response in mice given control antibody and the lower panels are sections from mice given agonistic anti-CD40 antibody. Note the large germinal centers in the control node seen as unstained areas surrounded by the IgD^{+} (brown-stained) follicular mantle (top left). These are totally absent in the treated mouse (bottom left). The massive numbers of CD11c^{high} dendritic cell (DC) (stained blue, bottom right) delineate the medullary cords. These are accompanied by large numbers of CD138^{+} plasma cells (blue, bottom left). By comparison, the extrafollicular response and the numbers of CD11c^{high} DCs in the medullary cords in the controls are relatively modest.

secretion. Studies of antibody production *in vivo* during extrafollicular antibody responses to viral infections show that neutralizing antibody is already secreted during the exponential growth of plasmablasts (85). Preliminary analysis of antibody responses in p18^{INK4c}-deficient mice showed a marked reduction in the number of antibody secreting cells. Surprisingly, when the extrafollicular response was analyzed in detail, the number of antibody-containing cells was not greatly altered compared to that in the response of wildtype control mice (86). Thus, there is a marked disparity between antibody-secreting cells and antibody-containing cells in these mice. In part, this difference is attributable to plasmablasts failing to come out of cell cycle and their premature death, but the loss of secretion is probably too great to be due to these factors alone. The possibility that secretion is being impaired by altered expression of proteins associated with secretion, secondary to compression of G1 is being explored. These findings are considered in more detail in the review by Dr Chen-Kiang in this volume.

Phase 4: long-lived plasma cells generated in extrafollicular responses

Most plasma cells generated during extrafollicular responses survive for approximately 3 days before undergoing apoptosis *in situ* (5, 87). Although most of the long-lived plasma cells generated during an immune response in the spleen and peripheral lymph nodes have been found in the bone marrow (4), there is good evidence that some of these are located in the spleen (39, 88, 89). A working hypothesis was set out in the previous section suggesting that association with CD11c^{high} dendritic cells is required for the transition of plasmablasts to plasma cells, which is tenable for short-term plasma cell survival but not for the proportion of splenic plasma cells that survive for extended periods. Immunohistology indicates that these long-lived plasma cells are no longer associated with CD11c^{high} DC, but they are located along the outside of red pulp blood vessels and collagen bundles that run between these vessels and the splenic capsule (39). Nothing appears to be known of the role of these structures, if any, in sustaining plasma cell survival. The splenic plasma cells that become long-lived appear to be chosen at random without reference to the isotype of antibody they produce, whether they are derived from germinal centers or extrafollicular responses or whether they have IgV-region mutations (39). Overall, the data are compatible with there being a limited numbers of niches in the red pulp that can sustain extended plasma cell survival. As a proportion of splenic plasma cells produced in all antibody responses become long-lived cells, it seems probable that there is a degree of competition between newly produced plasma cells and the residents of the niches. Such competition seems to be analogous to the gradual replacement of recirculating B cells by recruiting a small proportion of immature B cells to the pool (36).

Concluding remarks

The sophistication of mechanisms that regulate plasma cell differentiation from different types of B-cell precursors reflects the importance of neutralizing antibody in combating infection. Innate immune signals have key roles in the induction of these responses as well as through their influence on the DC that support plasmablast maturation and Ig class switching.

B1 cells, recirculating follicular cells, and marginal zone B cells (including memory B cells) can all be recruited into these early adaptive antibody responses. This wide-ranging recruitment may reflect the broad antigenic spectrum presented by the range of infections that evoke extrafollicular antibody responses. While early antibody production is potentially life saving, the usual immune response trade-offs apply between specificity, self-reactivity, and the time taken to generate a competent effector response. On a kinetic hierarchy, the primary extrafollicular antibody response is located in between innate responses initiated by cells expressing pattern recognition receptors, and memory responses by B cells expressing high affinity somatically-mutated receptors. Extrafollicular responses remain the source of adaptive humoral immunity against the accessible antigens on bacteria such as the pneumococci and meningococci that generally do not evoke T-cell help.

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