

Dendritic Cells, BAFF, and APRIL: Innate Players in Adaptive Antibody Responses

Minireview

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The first antibody produced in bacterial or viral infection results from B cell growth as plasmablasts. Dendritic cell-derived TNF-family ligands APRIL and/or BAFF enhance plasmablast survival and differentiation to plasma cells. Expression of these ligands by dendritic cells is promoted by innate immune signals that can convert subliminal B cell activation to a productive response. While this may be lifesaving in the face of infection, it can predispose to autoantibody production.

First line adaptive humoral defense involves antigen-induced activation of B cells to grow as plasmablasts and secrete antibody. A critical feature of these extrafollicular antibody responses is the relatively short lag phase between exposure to antigen and the onset of antibody secretion. This can be vital in limiting the spread of both viral and bacterial infections.

The Sequence of Events in Extrafollicular Antibody Responses

The following successive steps are associated with T cell-dependent extrafollicular responses (Figure 1). First, B cells that bind antigen through their B cell receptors are induced to move rapidly to the T zones of secondary lymphoid tissue and acquire a propensity to seek out primed T cells (Liu et al., 1991). Second, cognate interaction between these cells triggers the B cells to enter cell cycle and migrate yet again to either splenic extrafollicular foci (Jacob et al., 1991) or the medullary cords of lymph nodes (Luther et al., 1997). Third, the activated B cells differentiate to plasmablasts (proliferating cells with plasmacytoid features) which grow in these sites in the absence of T cells (Gulbranson-Judge and MacLennan, 1997; Luther et al., 1997) and further differentiate after about 3 days into fully secreting plasma cells (Sze et al., 2000). Many but not all of the plasma cells produced in these responses are short lived (Smith et al., 1996; Sze et al., 2000). T cell-independent antigens also trigger extrafollicular plasmablast growth, where the same sequence is followed without the intervention of T cells (Vinuesa et al., 1999b). The B cells move through four microenvironments during this response (Figure 1). In the first three of these, dendritic cells play important roles (Table 1). Extrafollicular responses contrast with the slower pathway to far more sustained antibody production, which is associated with

Ig V-region hypermutation and B cell selection in germinal centers. The two pathways to adaptive antibody production are not mutually exclusive and in most responses where T cell help is elicited they run in tandem. It has been suspected that accessory cells might also play a role in triggering B cells to grow as plasmablasts. Dendritic cells clearly do this through their role in priming T cells. In addition, data from studies in vitro indicate dendritic cells can influence B cell activation (Colino et al., 2002; Fayette et al., 1998; Litinskiy et al., 2002) and can transport intact antigen to B cells (Wykes et al., 1998). Previously, it has been shown that plasmacytoid dendritic cells, which are powerful producers of interferon- α (Asselin-Paturel et al., 2001), enhance antibody responses and induce nonselective Ig class switching (Le Bon et al., 2001). In this issue of *Immunity*, Balázs et al. provide direct evidence for the role of a subset of blood-derived dendritic cells in the transport of killed bacteria to B cells. They show that these dendritic cells are critical for a T cell-independent type-2 (TI-2) extrafollicular antibody response by promoting plasmablast survival. This effect is markedly impaired by neutralizing B cell activating factor (BAFF) and its closely related tumor necrosis factor family member APRIL (a proliferation-inducing ligand).

BAFF and APRIL in B Cell Development, Activation, and Survival

BAFF (Blys) and APRIL influence B cell maturation and survival at a number of levels. B cells are known to express three receptors for BAFF: BAFF-R, TACI (transmembrane activator and CAML-interactor), and BMCA (B cell maturation antigen); of these, TACI and BMCA also bind APRIL. BAFF and to a lesser extent APRIL are expressed in secondary lymphoid organs that support extrafollicular antibody responses, and BAFF expression is particularly associated with macrophages and dendritic cells. The expression and functions of these molecules have recently been reviewed in detail (Do and Chen-Kiang, 2002; Mackay and Browning, 2002; Rolink and Melchers, 2002). BAFF-deficient mice and mice deficient in BAFF-R have a block in maturation of transitional 1 to transitional 2 B cells (Schiemann et al., 2001; Yan et al., 2001a). This may in part reflect a requirement for BAFF and APRIL for continued survival of recirculating and marginal zone B cells, which are profoundly depleted within days of starting to neutralize BAFF and APRIL with soluble BMCA (Thompson et al., 2000) or TACI-Ig (Schneider et al., 2001). Impairment of signaling through TACI has been reported to be associated with reduced TI-2 and to a lesser extent T-dependent antibody responses (von Bulow et al., 2001; Yan et al., 2001b). Conversely, TI-2 responses are enhanced in mice overexpressing APRIL or BAFF (Stein et al., 2002).

During the early stage of B cell activation, in vitro BAFF signaling increases the numbers of antigen-activated B cells that survive to enter cell cycle (Do and Chen-Kiang, 2002). Also, both BAFF and APRIL induce nonselective Ig class switch recombination (Litinskiy et al., 2002) reminiscent of that induced by type-I interferons in mice (Le Bon et al., 2001).

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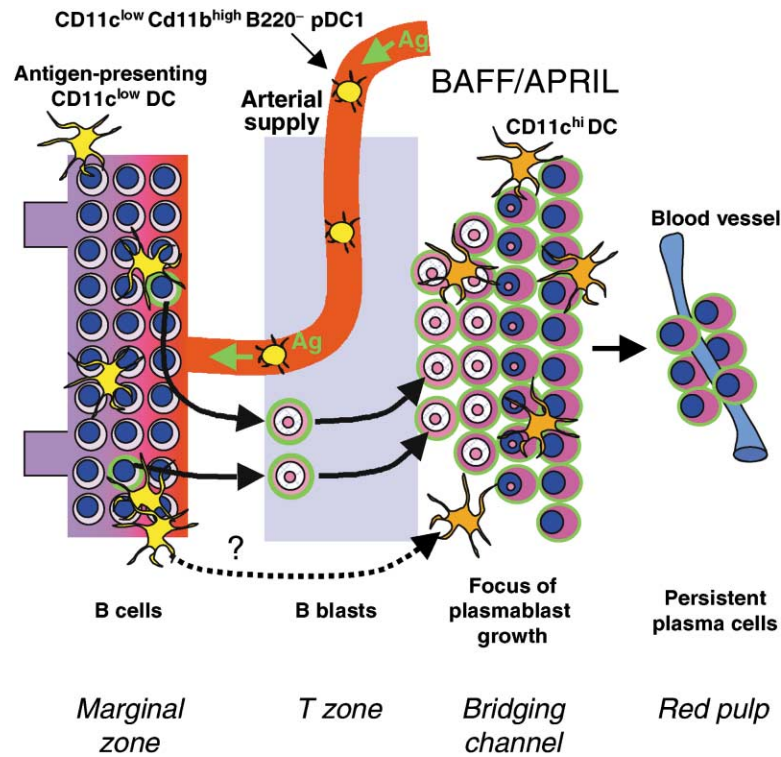


Figure 1. The Four Microenvironments of Extrafollicular Antibody Responses

When marginal zone or other B cells specifically bind antigen carried either free in the blood or transported by B220⁻CD11c^{low}CD11b^{high} blood dendritic cell precursors (pDC1), they rapidly migrate to the T zone. There, they are efficient at interacting with primed T cells and receive the signals that induce them to become plasmablasts. The plasmablasts migrate to extrafollicular foci where they proliferate in the presence of CD11c^{high} dendritic cells and, after about 3 days, differentiate into plasma cells. The splenic red pulp has a finite capacity to sustain a proportion of the plasma cells generated for extended periods. Dendritic cell involvement in each of these microenvironments is summarized in Table 1.

Induction and Control of Extrafollicular Antibody Responses

Much is known of the way B cells are activated by B cell-receptor engagement and T cell help. By contrast, relatively little is known of the signals that trigger B cells to grow as extrafollicular plasmablasts as opposed to B blasts, which proliferate in follicles and form germinal centers. Although both types of growth are frequently T cell dependent, there is no absolute T cell requirement to trigger either pathway of growth (Vinuesa et al., 2000). Once the signals for divergent differentiation are registered, key transcriptional regulators reinforce commitment to one or the other pathway of differentiation. Thus, differentiation to plasmablasts is regulated by expression of Blimp-1 (Mock et al., 1996; Shaffer et al., 2002),

which represses the expression of genes involved in B cell receptor signaling and proliferation while allowing expression of genes required for plasma cell development such as XBP-1 (Reimold et al., 2001; Shaffer et al. 2002). Those cells committed to form germinal centers upregulate Bcl-6, which in turn suppresses the plasmablast program of differentiation by repressing Blimp-1 (Fearon et al., 2001).

BCR engagement in the presence of cytokines (with or without the delivery of T cell signals) triggers the plasmablast reaction. Information is now emerging of factors that influence the control of plasmablast growth and survival and subsequent differentiation to plasma cells. Cell cycle arrest seems to be required for successful plasmablast differentiation to plasma cells. Blimp-1

Table 1. Dendritic Cell Involvement in the Microenvironments Associated with Extrafollicular Antibody Responses

Marginal Zone	Intact antigen is transported by monocyte-derived (B220 ⁻ CD11b ⁺ CD11c ^{low}) dendritic cells and is available to be bound by B cells. Marginal zone B cells are bathed by blood sinusoids; consequently, they also have access to free antigen in the blood.
T Zone	Dendritic cells with processed antigen have a critical role in priming T cells. There may also be a role for a range of dendritic cells in reinforcing B cell activation through their B cell receptors and cognate interaction with primed T cells, e.g., by BAFF and APRIL secreted by monocyte-derived dendritic cells. BAFF secretion can in turn be induced by type-I interferons produced by plasmacytoid dendritic cells, on activation by viruses and bacterial CpG.
Focus of Plasmablast Growth	Plasmablasts grow in association with the CD11c ^{high} dendritic cells that are typically found in this area. This is possibly the main site of BAFF and APRIL influence on plasmablast survival and their transition to become persistent plasma cells. T cells do not enter these foci.
Long-Lived Plasma Cells in the Red Pulp	These plasma cells are found lying along fibrous bands and contiguous blood vessels. The molecular and cellular basis of the signals that secure the survival of these cells is unknown. Plasma cells from extrafollicular foci compete with plasma cells coming from germinal centers for these sites.

directly represses *c-myc*, which is required for B cell proliferation and growth. It has also been shown that plasmablasts generated in mice deficient in the $p18^{lnk4c}$ CDK inhibitor form hyperproliferating antibody-containing plasmablasts that fail to differentiate into efficient antibody-secreting cells (Tourigny et al., 2002). The number of cell cycles completed by plasmablasts does not seem to be limited solely by stromal signals, for the end number of plasma cells produced in an unmanipulated extrafollicular response shows a direct correlation with the number of B cells recruited into the response (Sze et al., 2000). Thus splenic plasmablast growth in extrafollicular antibody responses of normal mice is largely restricted to the junction zones between the T zone and red pulp, while in mice with BCR encoded by a transgene, plasmablast growth can occur throughout the red pulp (Sze et al., 2000). By contrast, the capacity of the spleen to sustain late plasmablast or plasma cell survival is limited. Thus, in the situation where massive numbers of plasmablasts are generated in mice with transgenic B cell receptors, the number of plasma cells surviving more than 48 hr is no more than that in responses in wild-type mice where peak plasmablast numbers are far lower (Sze et al., 2000). Previous correlative studies suggest that the stromal element limiting plasmablast survival is a $CD11c^{high}$ dendritic cell population normally found in splenic extrafollicular foci and the medullary cords of lymph nodes (Vinuesa et al., 1999a). The mechanism of support was not identified. Balázs et al. (2002) now show that TACI-Fc-neutralizable molecules secreted by the dendritic cells might sustain late plasmablasts during their differentiation to plasma cells.

The precise role of BAFF and APRIL in enhancing the later phase of extrafollicular B cell responses has been difficult to assess since neutralization with soluble BAFF receptors has profound effects on total peripheral B cell numbers (Schneider et al., 2001; Thompson et al., 2000). Consequently, studies using short-term blockade of BAFF and APRIL once normal populations are established are required to test their effects on plasmablast growth and survival. Using such a strategy, Balázs et al. (2002) show that neutralization of BAFF (with an adenovirus-expressed TACI-Fc fusion protein) severely impairs antibody responses of mice to killed pneumococci. This results from impaired plasmablast survival rather than a defect in plasmablast generation or growth, because TACI-Ig expression does not impair the extrafollicular antibody response when Bcl-2 is overexpressed by the plasmablasts. The authors also show that the dendritic cell influencing plasmablast survival is a recent emigrant from the marrow that expresses low levels of $CD11c$ at the time of immunization. These cells probably correspond to $CD45RA^{-}CD11c^{low}CD11b^{+}$ blood monocytes with immediate dendritic cell precursor potential (pDC1) (Shortman and Liu, 2002). These cells have been shown to migrate into lymph nodes and produce mature dendritic cells upon phagocytosis of particulate antigens or culture with GM-CSF. They differ from the other subset of blood dendritic cell precursors, known as plasmacytoid dendritic cells (pDC2), which are also $CD11c^{low}$ but stain weakly with anti-B220 and anti-Gr1 (Asselin-Paturel et al., 2001). Plasmacytoid dendritic cells, when activated by viral antigens or bacterial CpG, have been shown to secrete large amounts of interferon- α , which

together with CD40 ligation and bacterial cell wall lipopolysaccharide is a potent inducer of BAFF expression on in vitro-generated dendritic cells (Litinskiy et al., 2002). This points to a hypothetical scenario in which bacteria induce plasmacytoid dendritic cells to produce type-I interferons, which induce BAFF and/or APRIL secretion from monocyte (pDC1)-derived DCs and their migration into secondary lymphoid tissues.

Balázs et al. provide evidence that $CD11c^{low}$, B220 $^{-}$ dendritic cells from the blood bind and transport the bacterial antigen to the marginal zone, where they make this accessible to B cells. Neutrophils and macrophages also take up the killed bacteria and accumulate in the spleen, but do not induce an antibody response. Since $CD11c^{low}$ antigen-transporting dendritic cells upregulate $CD11c$ on binding dead bacteria, they might be the precursors of the $CD11c^{high}$ cells found in extrafollicular foci. The de novo appearance of these dendritic cells at sites of plasmablast growth is shown by their appearance in previously rudimentary medullary cords during primary plasmablast growth (Vinuesa et al., 1999a). From Balázs et al.'s study, it is not clear whether the survival signal is delivered by the DCs at the time of antigen presentation to naive B cells in the marginal zone, or during contact with plasmablasts while they grow in the extrafollicular foci, or at both these stages.

The signal transduction pathway triggered by BAFF and/or APRIL that prolongs plasmablast survival is still not characterized. Impaired plasmablast survival is a feature of the response of Btk-deficient marginal zone B cells to TI-2 antigen. The antigen induces B cell translocation to the outer T zone and growth, but although the blasts start to upregulate Blimp-1 and cytoplasmic immunoglobulin, they die prematurely (Vinuesa et al., 2001). Btk plays a crucial role in BCR-dependent B cell survival through activation of NF- κ B (Bajpai et al., 2000), which is known to control the expression of Bcl-2 and Bcl-xL (Karin and Lin, 2002). It seems plausible that the signaling pathway that leads to NF- κ B activation is also involved in transmitting survival signals delivered by BAFF since BAFF can stimulate expression of Bcl-2 and Bcl-xL (Batten et al., 2000) and BAFF and APRIL can induce nuclear translocation of NF- κ B-Rel complexes (Litinskiy et al., 2002).

Although the enhancement of the extrafollicular response by BAFF and APRIL has so far only been confirmed in responses to T-independent antigen, these molecules may also augment and strengthen specific signals delivered during cognate interaction between T and B cells and sustain survival of extrafollicular plasmablasts generated during TD responses. T-independent B cell activation is crucial for early host defense against rapidly dividing pathogens and therefore occupies a place between T-dependent responses and innate immune responses. The findings of Balázs et al. (2002) provide fresh insight into the way in which TI-2 responses are regulated by innate immune responses. Innate augmentation of adaptive TI-2 responses may be lifesaving and hence selectively advantageous, but not necessarily risk free. Constitutive overexpression of BAFF in mice is associated with the development of autoantibodies and overt autoimmune disease (Mackay and Browning, 2002).

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