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Brief Reviews

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The Plasticity of Newly Formed B Cells

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Newly formed B cells (NF–B cells) that emerge from the bone marrow to the periphery have often been referred to as immature or transitional B cells. However, NF–B cells have several striking characteristics, including a distinct BCR repertoire, high expression of AID, high sensitivity to PAMPs, and the ability to produce cytokines. A number of findings do not support their designation as immature because NF–B cells have the potential to become Ab-producing cells and to undergo class-switch recombination. In this review, we provide a fresh perspective on NF–B cell functions and describe some of the signals driving their activation. We summarize growing evidence supporting a role for NF–B cells in protection against infections and as a potential source of autoantibody-producing cells in autoimmune diseases such as systemic lupus erythematosus. ***The Journal of Immunology*****, 2019, 203: 3095–3104.**

B cell subsets can be named based on where they reside. The B cells in follicles are follicular (FO) B cells. B cells in marginal zones (MZ) are MZ B cells. B cells in bone marrow (BM) are BM B cells, and the B cells in GALT are GALT B cells. Some B cells simply do not stay put and recirculate throughout the body. Many of the recirculating B cells are newly minted and have just left the BM. These newly formed B cells (NF–B cells) in some respects are like teenagers leaving home for the first time, young adults but not experienced adults. They have not yet gone through all of life's checkpoints to obtain a final specificity. They can be selected against and die young. Or in the presence of inflammatory or other signals, they can proliferate, secrete Abs or produce cytokines. In healthy humans, ∼40% of the Abs made by what Wardemann et al. (1) termed "immature B cells" are autoreactive (2). NF–B cells are present in the peripheral B cell pool throughout life, but are the most abundant peripheral B cell subset in neonates, before the naive B cell pool is established. NF–B cells are also the main peripheral B cell population in patients undergoing B cell–depletion therapy (3) and in some patients with immunodeficiency (4).

One widely accepted classification of B cells newly arriving to the spleen has been to define them as immature in contrast to mature FO or MZ B cells. The immature B cells in mice are surface IgM $(sIgM)^{++}$ and surface IgD $(sIgD)^{+}$ whereas the mature B cells are $sIgM^{+}sIgD^{++}$ (5, 6). A number of differences were identified between immature and mature B cells (6). Neonatal and immature B cells are particularly sensitive to clonal deletion or tolerance induction (7). Given the importance of defining how autoreactive B cells and Abs are selected against, the field has tended to focus on how NF–B cells are altered or selected to become FO or MZ B cells, rather than on the possible functions of the NF–B cells per se.

This review summarizes findings on NF–B cell selection and functions and the current understating of what factors are important for NF–B cell activation (Fig. 1). We have chosen the term "NF–B cell" as opposed to "immature B cell" because it more accurately denotes their status as recent BM emigrants, naive yet competent B cells.

NF–B cell development and selection in the periphery

After expressing a functional BCR on their surface, developing B cells in the BM may undergo rounds of selection through receptor editing or clonal deletion. Pre-B cells that escape negative selection in the BM develop into NF–B cells that enter the periphery and circulate through the blood or enter the splenic red pulp (RP) (5, 8). In mice, NF–B cells represent ∼15–20% of blood B cells and ∼10–15% of splenic B cells (9), although these percentages decrease with age. In humans, NF–B cells represent ∼4–10% of $CD19^{+}$ B cells in adult peripheral blood (10). The NF–B population in both mice and humans is heterogeneous. Several different classifications have been proposed to delineate different subsets of NF–B or transitional B cells (Table I). In general, mouse NF–B cells express high levels of sIgM, heat-stable Ag (CD24), and

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Abbreviations used in this article: AID, activation-induced deaminase; BAFFR, B cell–activating factor receptor; BM, bone marrow; bnAb, broadly neutralizing Ab; CSR, class-switch recombination; DC, dendritic cell; EF, extrafollicular; FO, follicular; GC, germinal center; HCV, hepatitis C virus; JDM, juvenile dermatomyositis; MZ, marginal zone; nAb, neutralizing Ab; NF–B cell, newly formed B cell; PB, plasmablast; PC, plasma cell; RP, red pulp; RTE, recent thymic emigrant; SHM, somatic hypermutation; sIgD, surface IgD; sIgM, surface IgM; SLE, systemic lupus erythematosus; TACI, transmembrane activator and CAML interactor; Tg, transgenic; WNV, West Nile virus.

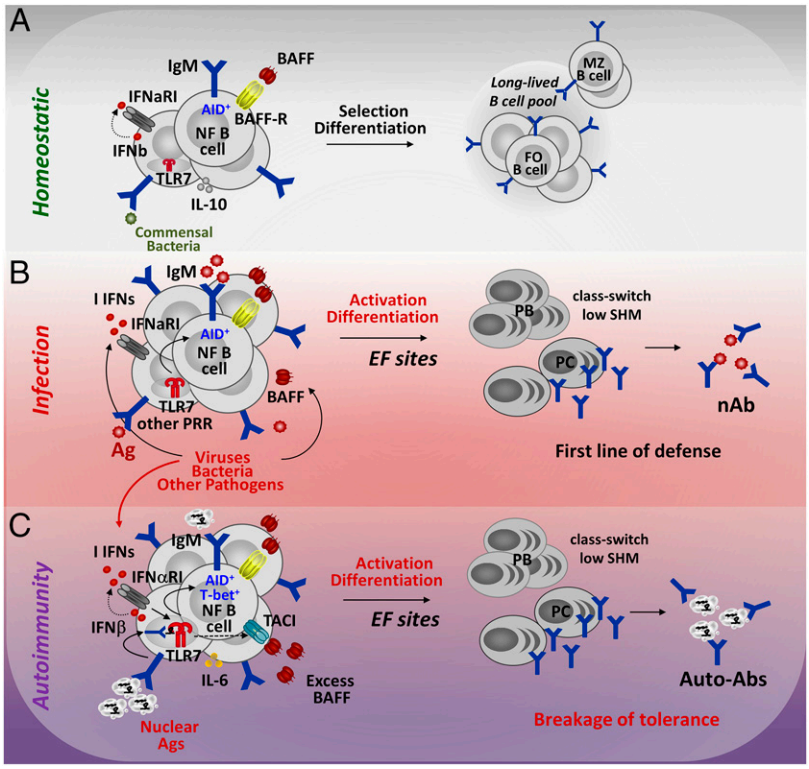

FIGURE 1. The fate of NF–B cells in the periphery. NF–B cells migrating to the periphery from the BM express AID and have a unique BCR repertoire, and 25–45% of NF–B cells are autoreactive. Under homeostatic conditions (**A**), NF–B cells undergo selection and become FO or MZ B cells. Their selection depends on signals received via the BCR, BAFFR, commensal bacteria, and other receptors. (**B**) Infections by viruses or bacteria lead to increases in type I IFN production and BAFF. NF–B cells recognize PAMPs (via pattern recognition receptors [PRRs]) and pathogen-associated Ags, become activated, and differentiate into PBs or IgG-secreting PCs, which produce nAbs. This provides a first line of defense against pathogens. (**C**) In autoimmune conditions, NF–B cell recognize self-antigens via the BCR, which, combined with signals through TLR7, IFN-αRI, and TACI (in the excess of BAFF), can drive direct activation of NF–B cells and the production of class-switched autoantibodies.

CD93. When they differentiate toward becoming FO or MZ B cells, they upregulate sIgD, CD23, and CD21 (8, 10–13). Human NF–B cells express sIgM and sIgD and are defined as $CD24^{hi}$, $CD38^{hi}$, $CD10^{+}$, and $CD27^{-}$ (14). As they become mature naive B cells, they downregulate sIgM, CD24, CD38, and CD10 and upregulate sIgD, CD23, and CD21 (3, 10, 14, 15).

NF–B cells in mice have rapid turnover and are subjected to both negative and positive selection (16, 17). Early studies suggested that NF–B cells cannot proliferate and undergo apoptosis upon BCR engagement. However, most of this work used strong BCR cross-linking as an Ag surrogate (7, 18, 19) without taking into consideration innate signals and the functions of NF–B cells per se, which, as discussed below, strongly depend on the settings where NF–B cells encounter Ags.

Under homeostatic conditions, some NF–B cells die, and others join the peripheral long-lived B cell pool (Fig. 1a). Just how and where NF–B cells are selected to become MZ and FO B cells is not entirely clear, even after decades of study [reviewed in more detail elsewhere (16, 17, 20)]. A current model suggests that NF–B cells are selected to become other B cell subsets or undergo apoptosis depending on the strength of the BCR signal or "BCR signaling threshold," which is established based on the cross-talk between signals received through the BCR and other secondary signals (20). An example of how other signals can affect NF–B cell fate is the cross-talk between BCR and B cell–activating factor receptor (BAFFR). Although BAFF promotes mainly mature B cell survival, signaling via BAFFR also plays a significant role in NF–B cell differentiation and survival (21–23). BCR and BAFFR signaling engage in complex cross-talk (24–27). In NF–B cells, BCR engagement drives the production of p100, which in turn is used by BAFFR signaling to promote cell survival (25). The expression of BAFFR on NF–B cells appears to require a tonic BCR signal (28). The absence of BAFF or BAFFR results in a reduction of peripheral B cells and a failure in B cell differentiation passed the NF–B cell stage (22, 28). BAFF transgenic (Tg) mice that overexpress BAFF, in contrast, have expanded peripheral B cells and develop systemic autoimmunity similar to human systemic lupus erythematosus (SLE) and Sjögren syndrome (29). This might be due to the rescue of autoreactive NF–B cells from negative selection (30, 31) or be associated with the activation of NF–B cells capable of class-switch recombination (CSR) and producing IgG autoantibodies (32, 33). Because BAFF levels can become elevated during infections and in some patients with autoimmune diseases, the effects of BAFF on NF–B cells are relevant to human disease. In addition to BCR/BAFFR cross-talk, NF–B cell selection and survival may depend on CD40 signaling (34, 35) or signals to endosomal TLRs (as discussed in more detail below).

Unlike FO and MZ B cells, NF–B cells constitutively express activation-induced deaminase (AID) (32, 36–38). This suggests that they may rapidly respond to Ags in vivo and undergo CSR or even somatic hypermutation (SHM) (36). Alternatively, Kuraoka et al. (39) and others (40) have found that AID must be expressed in NF–B cells for developing autoreactive B cells to be removed. Just how AID mediates this effect is not known. However, several groups have reported that TLR signals can upregulate AID in NF–B cells (32, 41).

Wardemann et al. cloned Abs from single B cells derived from the BM and blood of healthy donors and tested them for reactivity against nuclear and cytoplasmic Ags. About 40% of newly emigrated blood B cells (i.e., NF–B cells) react with more than one self-antigen (e.g., are autoreactive/polyreactive)

Table I. Characteristics of NF–B cells in comparison to other naive B cell subsets in the periphery

Cell Subsets	Surface Phenotype	Lifespan and Location	BCR Repertoire /AID expression	Possible Functions
NF–B cells	**Human**[a] sIgM^{hi}, sIgD$^{+/lo}$, CD10^{+}, CD27^{-}, CD24^{hi}, CD38^{hi}, CD21$^{-/lo}$ **(T1)** sIgM^{+}, sIgD^{+}, CD10^{+}, CD27^{-} CD24$^{+/hi}$, CD38$^{+/hi}$, CD21^{+} **(T2)** **Mouse**[b] sIgM^{hi}, sIgD$^{-/lo}$, CD93^{hi}, CD24^{hi}, CD21$^{-/lo}$, CD23$^{-/lo}$ **(T1)** sIgM^{hi}, sIgD^{+}, CD93^{+}, CD24^{hi}, CD21^{+}, CD23^{+} **(T2)**	Short lifespan Circulation Splenic Red Pulp (RP) Follicles (FO)	High autoreactivity AID^{+} (constitutive)	Precursors of mature naive B cells and short lived PCs Rapid, short lived Ab responses (T cell- independent) High sensitivity to PAMPs Productions of polyreactive nAb and autoantibodies Cytokine production Ag-presentation to T cells
Mature naive B cells	**Human**[a] sIgM$^{+/lo}$, sIgD^{+}, CD27^{-}, CD10^{-} CD24$^{+/lo}$, CD38$^{+/lo}$, CD21^{+}, CD23^{+} **Mouse**[b] CD93^{-}, sIgM^{+}, sIgD^{hi}, CD21 /CD35^{lo}, CD24^{lo}, CD23^{hi} **(FO)** CD93^{-}, sIgM^{hi}, sIgD^{lo}, CD21 /CD35^{hi}, CD24^{hi}, CD23$^{-/lo}$, CD1d^{hi} **(MZ)**	Long-lived Follicles (FO), Spleen or LNs Marginal zone (MZ), Spleen Circulation	Reduced autoreactivity AID^{-} (inducible)	Precursors of germinal center (GC), memory B cells, and short or long lived PCs Delayed and sustained Ab responses (T cell-dependent or independent). Production of hi-affinity protective Abs and autoantibodies Cytokine production Ag-presentation to T cells

[a]All cells in question are CD19^{+}, CD20^{+}.
[b]All cells in question are CD19^{+}, B220^{+}.

(1, 2). Martin et al. (42) isolated pre-B cells and B cells (sIgM^{+} CD38^{+}) from human BM and from the same donors, peripheral blood NF/transitional B cells (sIgD^{+}CD10^{+}CD27^{-}), and naive B cells (sIgD^{+}CD10^{-}CD27^{-}). Using long-read, high-throughput sequencing, they obtained and analyzed H and L chain gene sequences from these subsets. NF–B cells have a BCR repertoire different from the other B cell subsets and, importantly, are not simply precursors to naive B cells. This is pertinent in light of a recent report suggesting that the NF–B cell BCR repertoire may be shaped by signals and/or microbial symbiont Ags (43). The distinct BCR repertoire of NF–B cells may contribute to protection against pathogens. However, because NF–B cells are highly enriched for autoreactive/polyreactive specificities, they may also be a source of potentially harmful clones expressing autoantibodies.

Remarkably, the studies of Fink and her colleagues suggest that there is a T cell equivalent to the NF–B cell: recent thymic emigrants (RTEs). CD8 RTEs can rapidly respond to low-affinity ligands better than mature T cells and can quickly become effectors (44, 45). As with NF–B cells, the downside of RTE responses is that they can elicit autoimmunity. Like some NF–B cells, RTEs are tolerized without secondary inflammatory signals and express an anergy-associated gene signature (46).

NF–B cells in the protection against infections

Because NF–B cells have a distinct BCR repertoire, they are a potential unique source of Abs specific for pathogens. NF–B cells in humans and mice expand after viral or bacterial infections, suggesting they respond to pathogens and their products (Fig. 1b). Notably, patients with viral infections, including HIV and hepatitis C virus (HCV), have increased frequencies of circulating NF–B cells (4, 10, 47–50). A few in vivo studies assessing the role of NF–B cells during infections suggest they play an active role in protective immunity.

NF–B cells increase after viral infections. The frequency of NF–B cells increases during some viral infections, including HIV and HCV, and after influenza vaccine administration in children. HIV-infected patients with active disease have a significantly increased frequency of peripheral blood CD21^{lo}CD27^{-}CD10^{+} NF–B cells (47, 51). HIV immune responses are also characterized by the rapid expansion of atypical CD21^{lo}CD27^{+} IgG-producing plasma cells (PCs) and a reduction of memory B cells (47, 52). Because NF–B cells can class-switch, it is possible that the rapid forming IgG-producing cells in these patients develop directly from NF–B cells.

Furthermore, using a germline targeting strategy, Jardine et al. (53) showed that a population of human naive B cells that includes NF–B cells could be induced to undergo affinity maturation and produce broadly neutralizing Abs (bnAb) against HIV. Given that NF–B cells have a distinct repertoire from naive B cells (42) and apparently respond to commensal bacteria (43), they may have the potential to respond in a distinct way to pathogens like HIV. Whether germline BCRs found within the naive/NF–B cell repertoire can be cloned and modified to produce high-affinity bnAbs against HIV and other viruses is worth studying (53–56). The production of bnAb might be induced during polyclonal B cell activation, which occurs early after HIV and acute Ebola virus infection (57, 58).

The signals driving the expansion of NF–B cells in HIV patients are not known; however, HIV ssRNA activates TLR7 that can drive the expansion and activation of autoreactive NF–B cells (discussed below). HIV-infected individuals with advanced disease produce increased levels of autoantibodies (1, 59, 60). Because NF–B cells are enriched in autoreactivity, this raises the possibility that during HIV infection, NF–B cells become activated and produce Abs (47).

Chronic HCV infection is another example in which polyclonal B cell activation is observed coupled with the

expansion of NF–B cells. HCV$^+$ patients have increased frequencies of NF–B cells, which correlate with increases in serum BAFF levels (61). NF–B cells from HCV-infected patients compared with NF–B cells from healthy controls have a more proliferative phenotype (i.e., higher expression of the Ki67 marker). An increase in NF–B cells is also detected in children who have received influenza vaccinations (48). NF–B cell increases and activation might contribute to early and robust neutralizing Ab (nAb) responses and increased number of plasmablasts (PBs), particularly when a trivalent inactivated influenza vaccine is used (48). Thus, several studies report changes in NF–B cells after vaccination or infection with viruses known to induce early polyclonal B cell responses. NF–B cells respond to viral infection and presumably may play an active role in immune responses to viruses.

NF–B cells can provide protection against viral infection. Not many in vivo studies have assessed the role of NF–B cells during viral infections. NF–B cells can contribute to protective immunity against West Nile virus (WNV) in mice (62). Using a CD180-based immunization method that targets Ags directly to B cells, we induced strong and persistent humoral immune responses in BAFFR-deficient mice that have NF–B cells but lack FO and MZ B cells. After immunization, these mice develop nAbs and are protected from an otherwise lethal WNV infection. After WNV infection, NF–B cells in BAFFR$^{-/-}$ mice produce both WNV-specific IgG Abs and nAbs in the absence of germinal center (GC) formation. Furthermore, immunization of BAFFR$^{-/-}$ mice with the hapten NP attached to anti-CD180 induces the expansion of NP-specific NF–B cells and increases in NP-specific IgG Ab-secreting cells. Thus, NF–B cells can be induced to respond to specific Ags and can go on to make Ag-specific Abs in vivo.

Ag targeting to B cell subsets is a strategy to improve vaccine efficiency. After Ag is targeted to the CD180 receptor, which is expressed on all B cell subsets as well as other APCs, strong Ab and T cell responses are induced (63, 64). In mice inoculated with Ag conjugated to an anti-CD180 Ab (Ag–anti-CD180), NF–B cells, MZ B cells, and FO B cells process and present Ag and stimulate Ag-specific CD4$^+$ T FO helper cells (64). Within 24–48 h after immunization, Ag-specific B cells are detectable at T–B borders in the spleen, after which Ag-specific NF–B and FO B cells proliferate in a T cell–dependent manner. In contrast, Ag targeting to CD40 fails to activate NF–B cells and other B cells to become APCs, even though Ag-presenting dendritic cells (DCs) are activated. These results are consistent with previous findings that the consequences of Ag presentation by B cells are multifaceted and distinct from those of DCs. For example, the peptide/MHC class II complexes derived from BCR-associated Ag acquisition are predominately of the rare M1 MHC class II conformation, defined by the Ia.2 epitope (65). This MHC epitope is associated with the robust activation of CD4$^+$ T cells (66). Thus, the CD4$^+$ T cells activated by B cell APCs, including NF–B cells, may be qualitatively different from those stimulated by DCs. Furthermore, whereas with some Ags, initial CD4$^+$ T cell activation and expansion may rely on DCs, B cell APCs may be required for memory T cell formation (67). In some conditions, naive CD4$^+$ T cells can be activated directly by Ag-presenting B cells but not by DCs, such as following virus-like particle vaccination (68). The fact that NF–B cells can be induced to become APCs suggests that under some circumstances, NF–B cells contribute to protective T cell–dependent immunity. Further studies are required to define fully how and when NF–B cells are activated to become APCs.

NF–B cells respond to commensal bacteria. Chen et al. (43) reported that microbial symbionts influence host immunity by enriching frequencies of antibacterial specificities within the preimmune B cell repertoires early in life and in a T cell–independent manner. Mice colonized with microbiota for 21 d after weaning have increased frequencies of progenitor and splenic NF–B cells compared with their germ-free littermates. Importantly, changes in Ig repertoire (VH preference) induced by symbiotic microbiota evident in the naive B cell compartment are present in NF–B cells as early as 7 d after colonization. The exposure to microbial symbionts enriches the frequencies of antibacterial B cell specificities in the intestine and spleen (43). These results are in line with earlier findings by the Wesemann group (69) showing that primary B cell development in the intestine includes mucosal BCR editing and contributes to differential diversification of preimmune repertoires in the lamina propria and BM. The exposure to bacteria apparently enhances the diversity of the preimmune Ig repertoire of developing NF–B cells. This broadening of the NF–B cell BCR repertoire may enable hosts to respond to microbes rapidly and provide protection early in life. Commensal microbes influence the IgA repertoire to provide protection against bacterial infections (70–72). Wilmore et al. (73) showed that serum IgA and IgA secreting PCs in the BM elicited by a variety of commensal bacteria colonizing the gut protect against polymicrobial sepsis. Vossenkämper et al. (74) suggest that NF–B cell can migrate from the BM to GALT and differentiate into IgA-producing cells, providing protection against bacterial infections.

NF–B cells as a first line of defense against bacteria and parasites. NF–B cells can respond to bacterial stimuli. Capolunghi et al. (75) showed that cord blood NF–B cells respond to bacterial DNA through TLR9, express AID and Blimp-1, undergo SHM and CSR, and can produce antipneumococcal Abs. The ability of NF–B cells to respond to bacterial DNA may provide an important first line of defense early in life against pathogenic bacteria. NF–B cells and naive B cells transiently decrease after immunization of healthy individuals with the pneumococcal polysaccharide-based vaccine (64). NF–B cells and naive B cells also decrease early after vaccination, suggesting they may respond to bacterial products and turn into PCs. NF–B cells may also contribute to protection against neonatal sepsis (76). Elevated levels of circulating CD19$^+$CD38^{hi}CD24^{hi} NF–B cells correlated with protection. NF–B cells were significantly increased in survivors compared with healthy controls. In contrast, naive B cells (CD19$^+$CD38^{int} CD24^{int}) significantly decreased in all septic patients during the first 7 d postonset compared with healthy neonates, and their levels did not correlate with protection. The authors propose that NF–B cells may play a protective role in neonatal sepsis by releasing IL-10.

NF–B cells may also contribute to parasite-specific immune responses. *Plasmodium chabaudi* induces AID expression in NF–B cells and MZ B cells during acute infection in mice (77). NF–B cells differentiate into IgM- and IgG-secreting

cells in response to *P. chabaudi* infection in vivo, and in vitro in response to CpG. However, whether NF–B cells contribute to protection against malaria infection is still unclear. A recent study (78) showed that after acute infection of *P. vivax*, peripheral B cell subsets skew toward NF–B cells ($CD20^+CD21^+CD10^+CD27^-$), PCs, and atypical memory B cells. The increase in these B cell subsets in the acute phase of *P. vivax* infection reverts to baseline levels by day 30 when parasites have been cleared. *P. vivax* also induces a decrease in naive B cells and no changes in classical memory B cells. Both the NF and naive B cells appear to be activated. These changes in B cells after malaria infection result in parasite-specific and autoreactive IgM responses.

Children in malaria hyperendemic regions have increased levels of NF–B cells, together with increased levels of serum BAFF and inflammatory cytokines (79, 80). A recent study examined Ab repertoire development and diversification after malaria infection in infants and discovered unexpectedly high levels of SHM in blood B cells from infected infants as young as 3 mo old (81). Although infants have almost entirely naive and NF–B cells, after malaria infection, they have a degree of repertoire diversification comparable to that of B cells from adults after acute malaria infection. This study analyzed the repertoire of populations containing both NF and non-NF B cells. However, because NF–B cells express AID, it is possible that malaria infection induces SHM within the NF-B cell population.

Clonal selection of naive B cells rather than affinity maturation drives protective B cell responses to *Plasmodium falciparum* (82). With complex Ags, the efficiency of affinity maturation may not determine the quality of Ag-specific B cell responses, but rather the frequency of Ag-reactive precursors and their activation (82). Tan et al. (83) identified broadly reactive Abs that all carried an insertion of the LIAR1 domain and were common in malaria-endemic regions (84). It will be interesting to investigate if NF–B cells are a source of these broadly reactive Abs and contribute to protective immunity against malaria.

NF–B cells in autoimmunity

Increases in circulating NF–B cells are found in SLE and other autoimmune diseases, including type 1 diabetes and juvenile dermatomyositis (JDM) (49, 50). NF–B cells appear to play a key role in autoimmunity, and both TLR7 and type I IFN signaling are major drivers of their activation (85, 86) (Fig. 1C). Because NF–B cells are both enriched in autoreactive specificities and express AID, they may be an important source of pathogenic autoantibodies.

NF–B cell activation driven by signals to TLR7 and TLR9. B cells that recognize nuclear self-antigens receive second signals through the endosomal TLRs, TLR7 and TLR9 that recognize RNA and DNA motifs (87–89). Ligation of TLR9 with CpG drives AID expression and the differentiation of NF–B cells into IgM^+ memory B cells and natural IgM-secreting PCs (75). TLR9 may also drive NF–B cell differentiation toward autoantibody-producing MZ B cells (90). The role of TLR9 in B cell selection and activation remains unclear because the removal of TLR9 in lupus-prone mice results in worsen disease, which might be linked to the role of TLR9 in the regulation of TLR7-mediated B cell responses (86, 91). Consistent with this model, SLE patients have impaired TLR9 responses in NF–B cells (92, 93), whereas patients with severe disease have expanded NF–B cells, associated with high expression of TLR7 (94).

In mice, an increase in *Tlr7* dosage drives the development of lupus-like disease (85, 95–97) by promoting type I IFN production by myeloid cells and DCs and by driving autoreactive B cell activation. Whereas all peripheral B cells express TLR7, NF–B cells seem to be especially sensitive to signals via TLR7. H564Igi Tg mice (98) have rearranged Ig H and L chain genes specific for RNA/RNP-associated Ags. Some B cells in H564Igi mice undergo receptor editing and lose autoreactivity; the remaining BCR Tg (Id^+) B cells express markers suggesting they are developmentally arrested at the NF–B cell stage. Despite a presumed immature phenotype, Id^+ B cells in these mice produce class-switched (IgG2a and IgG2b) Abs (98). The development of autoantibodies in H564Igi mice requires TLR7 and AID expression in NF–B cells but is independent of T cell help (98, 99). NF Id^+ Tg B cells can also be induced in vitro to produce high levels of IgG Abs (32). Thus, data from this model suggest that NF–B cells contribute to TLR7-mediated autoantibody production.

*Tlr7*Tg mice with 8–16 extra copies of *Tlr7* gene (95) have a massive expansion and activation of NF–B cells in the splenic RP. Furthermore, we described that TLR7 overexpression leads to selective and rapid BrdU uptake in NF–B cells but not in FO B cells (32). The *Tlr7*Tg NF–B cells are hyperresponsive to TLR7 stimulation. The hyperproliferative phenotype of NF–B cells is associated with a further increase in AID expression and upregulation of T-box transcription factor (T-bet). Upon in vitro stimulation with a TLR7 ligand, NF–B cells produce high levels of IgG2b and IgG2c, including anti-RNA IgG. Although the effects of *Tlr7* overexpression on NF–B cell activation did not require IFN signals, the *Tlr7*Tg B cells we evaluated already expressed high levels of TLR7 (32). Other studies found that IFN signaling upregulates TLR7 expression in B cells, including NF–B cells, and is required for B cell responses to TLR7 stimulation (94, 100–103). The activation of NF–B cell in *Tlr7*Tg mice does not rule out the possibility that Ab-secreting cells in these mice also develop after FO B cell activation and GC formation, especially because *Tlr7*Tg mice have increased FO and GC B cells (32, 104). However, *Tlr7Tg* NF–B cells produce considerably higher levels of IgG Abs in response to TLR7 stimulation in vitro compared with *Tlr7Tg* FO B cells (32). The $CD93^+$ NF–B cells in *Tlr7*Tg mice are present in splenic RP. Thus, NF–B cells normally may become activated at extrafollicular (EF) sites. The relative contribution of NF–B cells as pathogenic autoantibody producers and whether activated NF–B cell interact with T cells in vivo needs further exploration.

NF–B cell dysregulation in human SLE and other autoimmune diseases. Studies in human SLE support a link between copy number variations and single-gene polymorphisms in the TLR7 gene locus and SLE susceptibility (105). $CD10^+CD24^{hi}CD38^{hi}$ NF–B cells are significantly expanded in $TLR7^{hi}$ SLE patients, of which the majority are TLR7 *rs3853839 G* risk allele carriers (94). Similar to the NF–B cells in the *Tlr7*Tg mice, NF–B cells obtained from $TLR7^{hi}$ SLE patients produce antinuclear IgG in response to TLR7 ligation in vitro, suggesting that human NF–B cells may be a direct source of autoantibodies. Elevated expression of *TLR7* in SLE

patients is also associated with an increase in IFN-α, which likely further contributes to NF–B cell activation and the overall disease activity in these patients. The TLR7^{hi} SLE patients developed a wide range of autoantibody specificities, which may well be due to increased survival and differentiation of autoreactive NF–B cells.

Other studies have reported increases in circulating NF–B cells in SLE patients (10, 106, 107). Wu et al. (108) found that newly diagnosed SLE patients have increased PTEN expression in B cells associated with the expansion of NF–B cells. Chang et al. (109) showed that IFN-α stimulation of NF–B cells from SLE patients induces increased Syk kinase activation after BCR stimulation. The expansion of NF–B cells has also been linked to increases in IFN-α levels and BAFF/APRIL production by neutrophils (110). Liu et al. (106) also explored the effects of IFN-α on NF–B cell survival, which leads to increased NF-κB activation and reduced expression of the proapoptotic molecule Bax. These changes in IFN responses and increases in NF–B cells may be driven by increased *TLR7* expression or signaling (94). Because overall B cell numbers may be decreased in SLE patients, the increase frequencies of NF–B cells could also be due to a loss of other B cell subsets and not necessarily reflect actual NF–B cell expansion. Nevertheless, newly diagnosed SLE patients and TLR7^{hi} SLE patients show an increase in NF–B cell frequencies and numbers (94, 108).

NF–B cells (again defined as CD10^{+}CD24^{hi}CD38^{hi} cells) are expanded in other autoimmune diseases, such as T1D (49). NF–B cells from pediatric patients with JDM actively divide and their frequencies correlate with disease activity. NF–B cells in JDM patients showed high IFN-α and TLR7 signatures and increase in IL-6 production (50).

NF–B cells are also increased in patients with X-linked lymphoproliferative immunodeficiency, some patients with common variable immune deficiency, and patients recovering from hematopoietic stem cell transplantation (4, 111, 112). Immunodeficiencies are frequently associated with systemic autoimmunity and the production of autoantibodies (113). Although this might be linked to defects in B cell selection in the BM, another possibility is the abundance of poly/autoreactive NF–B cells that in the absence of other B cell subsets may "fill the space" in the peripheral B cell compartment, as reviewed elsewhere (112). Kolhatkar et al. (114) showed an increase in NF–B cell activation and defects in NF–B cell selection in Wiskott–Aldrich syndrome patients associated with dysregulation of BCR and TLR signals.

Cytokine production and other effector functions of NF–B cells, related to autoimmunity. Another important function of B cells is cytokine production (115). NF–B cells in healthy individuals have been reported to mainly produce IL-10 and regulate T cells responses, consistent with regulatory B cell functions (116). However, NF–B cells in patients with SLE, rheumatoid arthritis, and JDM fail to suppress T cell responses and, compared with NF–B cells from healthy controls, produce less IL-10 and more IL-6 (50, 106, 116). This alteration of cytokine production by NF–B cells may be driven by type I IFN signals (94, 106), suggesting that under certain conditions NF–B can produce proinflammatory cytokines such as IL-6 and drive the activation of other immune cells.

Recent studies revealed another property of NF–B cells—an ability to produce type I IFNs (117, 118). Using a single-cell RNA seq approach, Hamilton et al. (103) showed that mouse splenic NF(T1) B cells constitutively express high levels of IFN-β. Furthermore, NF–B cells from BXD2 lupus-prone mice overexpress IFN-β compared with wild-type NF–B cells. In BM chimera experiments, NF–B cells lacking endogenous IFN-β express low levels of *Ifna* genes and *Tlr7* and have reduced survival and activation in vivo compared with IFN-β–sufficient cells. These results suggest that IFN-β is required for the optimal survival of and TLR7-driven responses of NF–B cells. Based on these data, the authors proposed a model in which endogenous IFN-β, via an IFN-β–TLR7–IFN-α loop, regulates NF–B cell development. Increased production of IFN-β production by NF–B cells may drive the induction of TLR7 and other type I IFNs during NF–B cell development, resulting in the loss of tolerance and increases in autoantibody production. Circulating NF–B cells and naive B cells in SLE patients express elevated levels of IFN-β, which correlates with disease severity (117). These new findings establish NF–B cells not only as a target of IFNs but also as producers of type I IFNs. However, they raise further questions as to what the signals are that regulate endogenous IFN-β expression in NF–B cells.

Because NF–B cells can function as APCs, it is possible they may present autoantigens and promote autoreactive T cell responses. Further studies are required to assess this.

NF–B cell activation driven by BAFF signaling. BAFF-Tg mice develop an autoimmune phenotype associated with the production of IgG2c and IgG2b autoantibodies. Although BAFF-Tg mice develop GCs, Groom et al. (119) showed that they can produce class-switched Abs even in the absence of T cells, suggesting that the activation of autoreactive B cells in these mice may occur in EF sites.

NF–B cells were only thought to express BAFFR; however, a recent study showed that NF–B cells in BAFF-Tg mice express detectable levels of another BAFF receptor, the transmembrane activator and CAML interactor (TACI) (33). Notably, the TACI^{+} NF–B cells are increased in BAFF-Tg mice. TACI^{+} NF–B cells are enriched in self-reactive BCR specificities, express AID and T-bet, and when cultured in vitro, spontaneously produced class-switched IgG Abs including antinuclear autoantibodies (33). TACI^{+} NF–B cells also produce greater levels of class-switched IgG autoantibodies ex vivo, as compared with MZ and/or FO B cells, suggesting that they may be a dominant source of autoantibody production in BAFF-Tg mice. The exact mechanisms driving TACI expression in BAFF-Tg NF–B cells and how TACI signaling promotes autoantibody production are not clear. Jacobs et al. propose that self-nucleic acids internalized via self-reactive BCRs may drive TACI expression. Because TACI shares signaling components (MyD88, TRAF5, and TRAF6) with the TLR pathway, TACI may cross-talk with TLRs (120, 121). BAFF signaling through TACI upregulates TLR7 expression in B cells (122). However, Du et al. (123) showed that the lack of TLR signaling does not affect the production of autoantibodies significantly and/or the expression of TACI on NF–B cells in the BAFF-Tg mice. The overall role of TACI in SLE remains somewhat controversial because some studies suggest that the expression of TACI inhibits B cell activation (124, 125). The studies of Jacobs et al. (33) and

Du et al. (123), however, suggest that TACI may play a different role in NF–B cells and help drive the formation of Ab-producing cells, particularly when BAFF levels are elevated.

Serum levels of BAFF are increased in patients with SLE, RA, SjS, or systemic sclerosis (126). However, the potential link between BAFF, TACI, and NF–B cells in humans requires further exploration. The deletion of TACI on B cells protects against BAFF-mediated autoimmunity in mice (122), suggesting a selective TACI blockade may be of therapeutic value in human SLE.

Conclusions

The collective properties of NF–B cells underscore the plasticity of this remarkable B cell subset (Table I). Clearly, NF–B cells can undergo selection in the periphery and contribute to an expanded B cell repertoire and protective immunity. A growing set of findings suggest they have important stand-alone functions in their own right and may play key roles in defenses against infections. NF–B cells not only express AID, have a distinctive BCR repertoire that can be shaped by commensal bacteria, and can undergo CSR, but also are highly sensitive to programming by type I IFN and TLR7 signaling. They can respond not only to commensals but also to foreign Ags and can become APCs. We propose that NF–B cells are an important innate B cell subset that contributes to rapid first line responses to infections (Fig. 1B).

EF B cell responses are responsible for the rapid Ab production postinfection and are associated with little or no hypermutation (127). During EF responses, AID is expressed, and developing PBs can undergo isotype switch independently of T cells (127–132). It is tempting to speculate that NF–B cells contribute to EF Ab responses not only because of the properties noted above but also because they are found in the RP. They may be key participants in EF B cell responses to infections and in the EF responses described in autoimmune mouse models and more recently in human SLE (133, 134). Infection by an attenuated strain of *Salmonella typhimurium* or by *S. enterica* leads to EF B cell activation associated with SHM and the production of *Salmonella* Ag-binding Abs (135–137). These studies did not determine which B cell subsets are involved in such responses. Further studies are required to assess if and when rapid EF responses and protective immune responses are driven by NF–B cells.

Tipton et al. (107) showed that a major fraction of Ab-secreting cell clones during SLE flares arise from naive B cells, rather than from GC or memory B cells (134). Although this study excluded NF–B cells, Wang et al. (94) reported that NF–B cells derived from SLE patients could become activated and produce antinuclear Abs upon TLR7 ligand stimulation. Data from *Tlr7*Tg, H564Igi, and BAFF Tg autoimmune prone mice further support EF activation of NF–B cells in the splenic RP (32, 33).

There are important implications if this remarkably understudied B cell subset can in fact be harnessed and programmed to contribute to immunity. First, possible roles for NF–B cells in immunocompromised patients may have been overlooked. Infants, the elderly, and immunocompromised individuals are at greater risk for severe viral infections but also are the most unresponsive populations to vaccination (138, 139). Thus, vaccination strategies that recruit NF–B cell and other B cell APCs, such as targeting Ags to CD180, may be particularly beneficial for these groups. The importance of NF–B cells in autoimmunity is emphasized by the fact that NF–B cells are enriched in autoreactive specificities and can become activated, particularly in response to innate signals (IFNs and TLRs) known to be major drivers of disease pathology, particularly in SLE (Fig. 1C).

The fact that RTEs have efficient trafficking to the periphery and a metabolism different from mature T cells (45) also suggests avenues for exploration of their B cell counterparts. Clearly, further studies are needed to define how recently minted lymphocytes can be programmed best to contribute to protective immunity. Perhaps NB T cells and B cells work together during initial immune responses.

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