

The Journal of Immunology

REVIEW ARTICLE | JANUARY 15 2019

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J Immunol (2019) 202 (2): 351–359.

<https://doi.org/10.4049/jimmunol.1801208>

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The Multifaceted B Cell Response to Influenza Virus

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Protection from yearly recurring, highly acute infections with a pathogen that rapidly and continuously evades previously induced protective neutralizing Abs, as seen during seasonal influenza virus infections, can be expected to require a B cell response that is too highly variable, able to adapt rapidly, and able to reduce morbidity and death when sterile immunity cannot be garnered quickly enough. As we outline in this *Brief Review*, the influenza-specific B cell response is exactly that: it is multifaceted, involves both innate-like and conventional B cells, provides early and later immune protection, employs B cells with distinct BCR repertoires and distinct modes of activation, and continuously adapts to the ever-changing virus while enhancing overall protection. A formidable response to a formidable pathogen. *The Journal of Immunology*, 2019, 202: 351–359.

The B cell-derived Abs generated in response to influenza virus are critical for preventing death from acute respiratory tract infections, promoting a more rapid and full recovery, and providing continued protection from future infection-induced illness and/or death. This is despite the fact that the yearly re-emerging seasonal strains of influenza virus undergo rapid point mutations, “antigenic drift,” which reduces the effectiveness of strain-specific Abs generated from 1 year to the next (1). Nonetheless, pre-existing, nonneutralizing humoral immunity, even if unable to prevent reinfection, can prevent serious disease and/or reduce mortality. This is well illustrated by the fact that individuals most at risk from dying following influenza infection are the very young and old or those with immunodeficiencies, patients in whom an effective immune system has either not developed or is compromised. In addition to the mostly strain-specific Abs generated following an infection, influenza-specific B cell responses can include rare responses against highly conserved antigenic epitopes. Responses to such epitopes can provide cross-protection against multiple influenza strains (i.e., induce “heterosubtypic” influenza-specific immunity). The specificity of these cross-protective Abs and their protective capacities has been a recent focus of

anti-influenza vaccine development efforts and are reviewed in detail elsewhere (2).

Advances in our understanding of B cell responses to infections have revealed their complexity. In addition to the generation of Abs, B cells also generate cytokines with which they can regulate the immune response, and they can act as APCs to CD4 and CD8 T cells (3). Their elaboration of cytokines and interaction with T cells is likely to induce responses that are distinct from those provided by other APCs, shaping T cell immunity in yet to be determined ways. Moreover, the presence of virus-induced innate responses can also affect B cell functions, and this may differ depending on the tissue in which B cells receive such signals, thus indicating further complexities based on B cell tissue location and interaction with innate immune cells. Further complexities are the extensive heterogeneity among B cells with regard to their developmental origins, initial BCR affinity for influenza Ags, and their differentiation state, all of which shape B cell response outcomes.

In this article, we review the multiple aspects of B cell immunity to influenza virus infection, focusing on experimental mouse models and citing human studies when possible. We discuss the role of B cell responses to innate signals and B cell-derived cytokines involved in antiviral responses as well as describe how B cells transform these signals into functional changes. We review innate-like B cell responses, early extrafollicular (EF) plasmablast, and later germinal center (GC) responses and argue that the latter two are of equal importance in the development of strong and durable humoral immunity to influenza virus infection. Understanding the complexities involving B cell responses to influenza infections may help to overcome the challenges of inducing long-term immunity through vaccination.

B cells shape the early immune response to influenza infection

Mice never exposed to influenza virus and even those kept under germ-free conditions nonetheless have circulating “natural” IgM Abs, a fraction of which can bind to a variety of different influenza virus strains (4–6). This IgM Ab is generated in the bone marrow and spleen of mice by differentiated, neonatally derived subsets of innate-like B cells called B-1 cells (6, 7) and can reduce viral loads and mortality rates

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Received for publication August 30, 2018. Accepted for publication October 17, 2018.

J.H.L. is supported by National Institutes of Health (NIH)-T32 training Grant HL007013. Recent work from this laboratory described in the review was supported by NIH/National Institute of Allergy and Infectious Diseases Grants U19AI109962 and R01AI117890.

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Abbreviations used in this article: ADCC, Ab-directed cell cytotoxicity; ASC, Ab-secreting cell; BALT, bronchus-associated lymphoid tissue; B_{mem}, memory B; DC, dendritic cell; EF, extrafollicular; Fc_μR, Fc receptor for IgM; GC, germinal center; medLN, mediastinal lymph node.

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from infection (8, 9). In addition to this steady-state function, innate-like B-1 cells also respond to influenza virus infection with rapid migration from the pleural cavity to the draining mediastinal lymph nodes (medLN) (5, 10). Accumulation in the medLN, which occurs as early as 2 d after influenza infection, depends on type I IFN production in the respiratory tract, which was shown to cause integrin CD11b activation on the surface of pleural cavity B-1 cells, facilitating their retention in the medLN (10). Once there, some B-1 cells begin to produce IgM, including IgM that can bind multiple strains of influenza virus (4, 5). The mechanisms leading to induction of Ab secretion by these cells remains unknown.

Secreted IgM is a potent facilitator of complement activation (11) and can bind the complement receptors (CR1/2) as well as the Fc receptor for IgM (Fc_μR) expressed on dendritic cells (DCs), macrophages, and B cells (12, 13). Lack of IgM in mice led to delayed influenza clearance (8, 9, 14, 15). IgM-mediated protection from influenza infection is mediated at least in part through activation of complement (15). In addition, lack of secreted IgM and/or lack of Fc_μR expression on B cells caused decreases in influenza-specific IgG, likely further contributing to reduced viral clearance (9). Thus, early production of IgM in response to influenza optimizes the overall Ab response and is a potential catalyst in priming both innate and adaptive immunity.

Immediate early responses by bone marrow-derived conventional follicular B cells (also termed B-2 cells), which make up the vast majority of B cells in lymphoid and nonlymphoid tissues, are less well characterized. Some potential functions may be inferred from other infection models. In addition to populating secondary lymphoid tissues, naive B cells are dispersed throughout the conductive airways and lung parenchyma at homeostasis (16) and are thus able to respond rapidly to respiratory infections. In support of this, lung-associated B cells were observed transporting Ag to the spleen, but not the lymph nodes, within 2 h after intranasal immunization of mice with virus-like particles (17). Following intratracheal administration of anthrax spores, B cells were found to deliver Ag within just 6 h to the lung-draining lymph nodes in a BCR-independent manner (18). These observations indicate early functional roles of B cells in Ag delivery from the periphery to secondary lymphoid tissues. The nature of the Ag and/or the inflammatory responses induced to distinct pathogens may affect subsequent localization of these B cells and therefore the initiation of the adaptive response.

B cell responses during influenza infection are present in both medLN and spleen. However, splenic responses appear to be minimal and of shorter duration compared with those in the medLN, indicating that the medLN are the main sites of B cell response activation after intranasal influenza infection, at least in experimental settings. In humans, where severe infections usually proceed from the upper to the lower respiratory tract, the involvement of upper respiratory tract draining cervical lymph nodes likely play a greater role compared with experimental infections of mice, in which the virus is usually applied deep into the respiratory tract.

The medLN significantly increase in size and cellularity within 1–2 d postinfection (19). Influenza Ag-carrying CD11b⁺ DCs migrate into these lymph nodes, thereby promoting the activation of CD4 and CD8 T cells. Interestingly, lymph node expansion following lymphocytic choriomeningitis

virus infection was shown to depend on the provision of lymphotoxin $\alpha 1\beta 2$ by B cells (20). Furthermore, 2 d after immunization with the Ag keyhole limpet hemocyanin in CFA, B cells facilitated a 6-fold increase in lymph node cellularity by secreting vascular endothelial growth factor A (VEGF-A) (21), which promotes both blood and lymph vasculature remodeling and growth (22). Without B cells or B cell-derived VEGF-A, DC trafficking and lymph node cellularity were reduced to homeostatic levels (21). Thus, B cells actively regulate immune responses by shaping secondary lymphoid tissue remodeling postinfection.

Within the lymph nodes, B cells become subjugated to cues for activation provided by innate signals. Global gene expression analysis on medLN B cells of day 2 influenza-infected mice revealed a type I IFN signature (23). Type I IFN was shown to cause rapid global B cell activation, likely prior to first Ag encounter, including immediate upregulation of TLR3 and 7 and increases in expression of CD69 and CD86 (23). This renders B cells more responsive to innate signals and increases their ability to interact with T cells by trapping them in the lymph node as well as increasing expression of costimulatory molecules. Indeed, these innate signaling-derived activation signals were shown to be important for B cell response induction, as B cell-specific deletion of the type I IFN receptor diminished the influenza-specific Ab response as early as 3 d postinfection (23). Thus, adaptive B cell response activation is integrated in, and dependent on, the network of infection-induced innate signals. Further exploration of how innate activation of B cells affects the quality and quantity of anti-influenza responses will be important for the development of adjuvants that support stronger and longer-lasting humoral immunity.

B cell activation leads to rapid induction of EF and slower development of GC responses in draining lymph nodes after influenza infection

As discussed above, respiratory tract draining lymph nodes are the main site of influenza-induced B cell activation. A critical outcome of B cell activation is the induction of Ab-secreting cells (ASCs) producing class-switched, protective Abs (24, 25). Strong influenza-specific Ab responses are produced by EF B cells well before GC-derived plasma cells are generated (Fig. 1). EF responses are situated in the lymph node medulla, where rapid and strong B cell proliferation is followed by differentiation of these cells into short-lived plasmablasts that secrete IgM, IgA, and IgG (26).

As we explore below, the explicit mechanisms that drive B cells into either the EF or the GC fate have not yet been completely resolved. Of importance, the kinetics of the EF response correlate with the peak and resolution of primary influenza infection (27), indicating that this rapid, strong, and early humoral response contributes to virus clearance. In contrast, GC responses do not usually form until the infection has nearly resolved, indicating GCs are geared toward generating and maintaining immunological memory by producing long-lived Ab-secreting plasma cells and memory B (B_{mem}) cells. The latter importantly enhances the precursor frequency of influenza-specific B cells, some of which can respond to the next influenza virus challenge either by initiating a rapid EF response or by forming new GCs (Fig. 1).

Apart from the above-discussed migration of Ag-carrying B cells into secondary lymphoid tissues, most B cells likely come across Ag in the lymph node follicles through stochastic

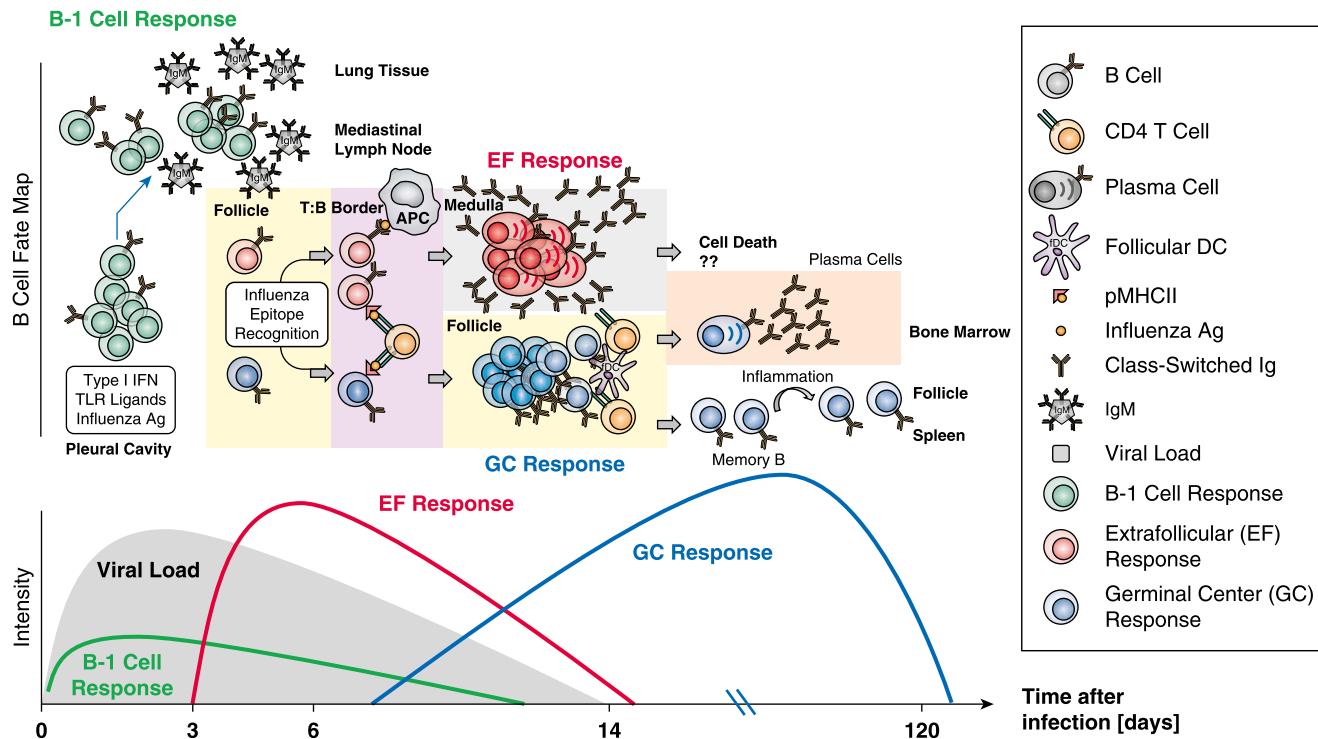


FIGURE 1. The heterogenous nature of B cell responses to influenza virus infection. Top, B-1 cells, activated by various innate signals, including type I IFN, respond to initial influenza infection with migration to the draining medLN, where these cells differentiate to IgM-producing cells. Conventional B cells, activated by influenza Ag, will migrate to the T-B border, where they receive “T cell help.” Thus activated, B cells will differentiate along one of two pathways: EF, which induce strong and rapid clonal expansion and differentiation to Ab-secreting plasmablasts, and GCs in the B cell follicles. EF-derived plasmablasts are thought to live for only 3–5 d, whereas the outcome of GC responses is the development of long-lived Ab-secreting plasma cells in the bone marrow and circulating nonsecreting B_{mem} cells. Inflammatory signals may activate these B_{mem} cells to rapidly migrate to spleen and lymph nodes and to differentiate to ASCs or undergo new diversification in GC responses. Bottom, B-1 and EF responses are the only B cell responses that are sufficiently fast to influence viral clearance after a primary challenge.

movement that bring them near the follicle’s capsular plane, where they encounter Ag tethered on subcapsular macrophages (28). B cells can also find cognate Ag elsewhere in the lymph node (reviewed in Ref. 29), although whether B cell response quality differs depending on the type and site of Ag encounter is unknown. The Ag-specific activation of B cells and CD4 T cells induce genetic reprogramming that strongly promotes their interaction, specifically their chemokine-mediated migration toward each other. This is facilitated initially by the upregulation of CCR7 and downregulation of CXCR5 in B cells and the inverse in CD4s, which leads to their congregation at the paracortical ridge (30), otherwise known as the T:B border. Movement of B cells is also affected by the orphan receptor Ebi2, which is expressed differentially at the many stages of B cell activation and differentiation in the lymph node (31). Furthermore, Ag stimulation induces secretion of CCL3 and CCL4 by B cells, which will further attract activated CCR5-expressing CD4 T cells and thus enhance T-B interaction and humoral immunity (32).

EF responses. Ag-activated B cells can form EF foci independent of CD4 T cell “help,” and such T cell-independent B cell responses can confer some protection against influenza virus infection (33, 34). However, most protective B cell responses to influenza, including those generated in EF responses, are T cell-dependent. The local expansion and rapid differentiation of B cells into plasmablasts, seen in the EF response, requires B cell genetic programs to polarize toward an ASC fate. The ASC state is characterized by expression of transcription factors Blimp1, XBP1,

and the high expression of IRF4, which is upregulated upon initial B cell activation together with IRF8. However, IRF4 and IRF8 become mutually antagonistic after reaching certain expression thresholds, influencing polarization toward an effector (IRF4) or a GC (IRF8) fate (35).

The signals that preferentially induce IRF4 over IRF8 and thereby an ASC fate are the subject of ongoing investigation. Elegant previous work has begun to shed light on some potential mechanisms. Of particular significance, a strong correlation was observed between BCR affinity for Ag and the ASC fate (36–38). Reducing a high-affinity interaction of B cells to the model Ag hen-egg lysozyme, by alterations made to the cognate Ag, showed that only high-affinity BCR–Ag interaction could promote EF responses (36). This is supported also by *in vivo* studies showing that GC-derived, B_{mem} cells will rapidly form EF responses during a recall response preferentially over generating new GCs (39). At the molecular level, and consistent with those observations, IRF4 is known to be upregulated by increasing affinities of the BCR for Ag. Furthermore, IRF4 is induced in a dose-dependent manner by the engagement of B cell-expressed coreceptor CD40 with CD40L on T cells (40). These observations strongly suggest that BCR signal strength, as well as T cell help, contribute toward differentiation of activated B cells into plasmablasts. Whether strong BCR signaling alone can overcome the need for CD40 engagement in the formation of T cell-independent EF responses or whether IRF4 induction might be induced by innate signals in addition to BCR signaling remains to be determined.

A model of rapid EF response induction that depends on the presence of high-affinity BCR is consistent with data demonstrating that these responses can provide Ab rapidly and of sufficient quality to contribute to immunity after influenza infection (27). However, it also suggests that rapid EF responses are formed only when B cells of “sufficient” Ag affinity are present in a given host prior to an infection. The high morbidity and mortality rates observed in newborns and the very young after influenza infection might be in part explained by the fact that these individuals lack such a robust and broad naive B cell repertoire (41) and that they do not yet carry influenza-specific B_{mem} cells that could provide clones specific for influenza (see also below).

Once an activated B cell differentiates into a plasmblast, their populations begin to expand rapidly in the lymph node medulla and secrete predominantly class-switched Ab, peaking between 7 and 14 d after influenza infection (27, 42). In humans, influenza-specific plasmablast numbers in the peripheral blood peak around 7 d after symptom onset during infection (43) and after immunization (44). However, post-immunization plasmablasts appear at a much-reduced frequency compared with infection (45) and have even higher levels of somatic hypermutation than circulating B_{mem} cells (46), indicating they were recruited from memory pools and thus previously generated in GC rather than EF.

Once an EF response is initiated, its maintenance does not appear to require further T cell help (47). Interestingly, it was shown that an increased presence of neutrophils in the draining lymph nodes led to increases in plasmablast numbers after s.c. CFA immunization (48). The mechanisms of that interaction were inferred to be dependent on the cytokine BAFF secreted by neutrophils. However, other studies more explicitly showed that the survival factor A proliferation-inducing ligand (APRIL), also secreted by neutrophils, was vital in maintaining ASC populations within niches of the lower respiratory tract submucosa (49) as well the splenic marginal zone (50). Plasmablasts from the EF response thus seem to be maintained or at least significantly supported by Ag-independent means, including through the secretion of survival factors by effectors of the innate compartment.

GC responses. GC-derived expansion of B cell clones is minimal during initial influenza infection and virus clearance (i.e., the first 7 to 10 d after virus exposure). Instead, GC-associated B cell numbers peak well into the contraction phase of the immune response (51) (Fig. 1). To initiate GC formation, B cells activated by Ag and cognate CD4 T cell help return to the B cell zone (follicle) from the T:B border, along with their cognate CD4 T cells, in which follicular DCs display nonprocessed Ags (52). Ag-binding B cell clones then undergo massive proliferation as well as somatic hypermutation, the latter supported by the expression of the enzyme *aicda* (AID), forming the dark zone of the GC with cells known as centroblasts. This process leads to B cell subclones with genetic alterations in the Ag-binding site of their BCR. Centroblasts then migrate to the light zone of the GC for validation of Ag specificity. Once there, these cells, known as centrocytes, engage with CD4 follicular helper T cells to determine B cell fate (53). Positive selection will result in either a return to the dark zone for further rounds of proliferation and mutations or in an exit from the GC as either B_{mem} cells or terminally differentiated plasma cells. It is

not known what signals instruct the differentiation of GC clones to either a B_{mem} or plasma cell fate, but lower-affinity clones were shown to preferentially accumulate within the B_{mem} pool (54–56), whereas high-affinity clones are shunted preferentially toward a plasma cell fate (25–27) in much the same manner as seen during the EF response. Therefore, it appears that both plasmablasts and post-GC plasma cells may use the similar differentiation pathways to reach an ASC state. However, although EF-derived ASCs appear to be short-lived and confined to the site of their induction, the post-GC plasma cells migrate to the bone marrow in a CXCR4-dependent manner. There, they can secrete Ab and survive long term, supported by local, stromal cell-derived survival signals (57).

Extensive Ig sequencing studies of circulating B_{mem} cells in humans have shown that strong Ab repertoire diversity is generated in response to repeated infection with, or exposure to, seasonal influenza viruses. The data suggest similar strong, ongoing GC responses to occur in humans following influenza infection as those studied in experimental mouse models (58). Given that both the influenza virus and the B cells are relying on mutations to out-compete each other, the GC response is likely the main B cell response engaged in the “arms race” between the virus and the humoral response, as somatic hypermutation in EF responses is limited (59, 60). Indeed, sequencing studies have been able to trace the evolution of B cell responses that occur in response to repeated exposures to seasonal influenza virus strains (61–63), clearly revealing a GC-derived B_{mem} cell repertoire that is shaped by repeated exposures to mutated viruses.

Humoral immunity to influenza integrates the B-1 response and B-2-derived EF and GC responses

Thus, humoral immunity to influenza can be categorized into immediate early, early, and late responses, each facilitated by a distinct set of B cells. Control of an acute influenza infection by B cells involves both innate-like B-1 cells as well as rapidly responding conventional B cell-derived EF responses, both having the capacity to control early tissue virus load. This control is critical as an early containment of this rapidly dividing virus can reduce the risk of overshooting cytokine and CD8 T cell responses, which can cause extensive and excessive accumulation of leukocytes in the lung parenchyma, lung pathology, and eventual organ failure.

GC B cell responses, in contrast, must fulfill distinct roles in combating influenza infection. The kinetics of these responses precludes important roles during primary infection. Yet the induction and then maintenance of GC responses for months after the clearance of the virus suggests that their main function is the strengthening of the initial line (i.e., B-1 and EF) of humoral defense during recall responses, either through generation of Ab-producing plasma cells or through provision of a large repertoire of B_{mem} cells. The production of affinity-matured, neutralizing Abs by plasma cells per se seems of limited value against an infection that can rapidly mutate targeted epitopes. Therefore, the generation of a broad repertoire of B_{mem} cells seems very important as these B cells are generated to a variety of influenza Ags and can respond, depending on their initial affinity to Ag, by either rapidly generating Abs or by inducing strong GC responses. It may also explain why such B_{mem} cells are not of as high affinity for the inducing Ag as the effector plasma cells; the next round of

infections might bring mutated Ags that bind differently to these BCR. Recent studies with human B_{mem} cells support the notion that this compartment has a broad repertoire and includes cross-reactive BCR-bearing cells that are continuously shaped by repeat exposure to seasonal influenza virus strains (58, 64). The broadening of this repertoire would ensure that there are B cell pools available to bind viral Ags, even if altered by mutations, as seen during the seasonal encounters with influenza by humans.

Although EF and GC responses to influenza are generated by conventional B cells, early sequencing studies showed a remarkable lack of overlap in the V-gene usage of hemagglutinin-specific B cells early and later after immunization with influenza virus (27, 65). Recent elegant studies by the Yewdell group (51) confirmed these early studies using influenza virus infection of mice. The data are consistent with the above-discussed findings that only high-affinity Ag–BCR interactions drive EF responses (36), whereas lower-affinity interactions initiate GC responses. Given the strength of the EF response postinfection, this suggests that humoral responses to influenza virus infection, and likely other infections, do not begin with the elaboration of low-affinity Abs that, over time, affinity mature into higher-affinity Ab responses. Rather, high-affinity Ab responses produced by the EF response are replaced later by high-affinity Ab responses generated from the GC response.

If our analysis is correct, this would be a significant departure from the textbook idea of Ab responses that “mature” over time but very consistent with conclusions drawn from previous studies with vesicular stomatitis virus infection of mice that failed to demonstrate overall changes in serum Ab affinity over the course of that virus infection (66). For an acute infection, like infections with influenza virus, the immediate early activation of B cells into ASC from pre-existing high-affinity clones may make the difference between life and death, although during more protracted infections the influence of the EF response may be more modest.

Respiratory tract B cell activation during influenza infection

In addition, and similar to other mucosal sites, strong and sustained respiratory tract tissue injury, including injury induced by influenza infection, can result in the formation of bronchus-associated lymphoid tissue (BALT) in the submucosa of the upper respiratory tract and within the parenchyma of the lower respiratory tract airways (67–69). Although the importance of BALT formation in immune defense is still not resolved, such inducible BALT was shown to contain GCs and to generate influenza-specific Ab responses after influenza infection in mice that lacked all secondary lymphoid organs, including all draining lymph nodes (67), indicating their potential to catalyze adaptive T and B cell responses. Interestingly, following local ablation of BALT with diphtheria toxin, significant reductions in influenza-specific serum Ab titers were noted after intranasal influenza inoculation (70). However, another study showed that after influenza infection, BALT formed only if mice had received repeated intranasal administration of LPS as neonates (71). Differences in virus strain and mouse models may be the cause of these discrepancies. Together, the data indicate that tertiary lymphoid tissues in the respiratory tract may contribute to immunity during strong and/or chronic immune activation.

The GC generates ASCs and B_{mem} cells capable of recall responses and long-term influenza-specific Ab production

Following the resolution of influenza infection, ASC populations are found long term in bone marrow as well as locally in the respiratory tract (72). The differentiation pathways of the local ASCs have not been resolved, although those residing in the bone marrow likely arise from GC responses. Both influenza infection-induced bone marrow and lung ASCs were shown to require TACI (a receptor for APRIL) for long-term survival, whereas lymph node and spleen ASCs were unaffected by the loss of that receptor (73). Given that lymph node ASCs are derived mainly from EF responses, as they disappear with the resolution of the EF response and before robust GC responses develop (42), this could suggest that cell-fate decisions between plasmablasts and plasma cells seem to be the result of broadly overlapping genetic programs. Differences might still exist between individual ASC populations based on tissue location and/or whether their differentiation pathways involved EF or GC responses.

GC-derived plasma cell development leads to sustained influenza-specific serum Ab titers. Ag-specific Ab has been detected decades postinfection and sometimes after immunization in humans (74). Carbon dating on plasma cells in the gastrointestinal tract confirmed this subset’s aging capacity (75). Similarly, survivors of the 1918 influenza pandemic showed seroreactivity to the 1918 virion 90 y later (74). This indicates that influenza infection can generate essentially life-long humoral protection against the same (homosubtypic) virus strain. Whether boosting by subsequent seasonal influenza exposures contributes toward maintenance of such Ab responses is not known and should be considered.

Few data are available revealing the lifespan and aging dynamics of B_{mem} cells. It has been well demonstrated, however, that these cells respond acutely to secondary challenges, partaking in both EF and GC responses in local lymph nodes (39, 76), as well as migrating directly to sites of insult (77, 78). More than 5 mo after influenza challenge, class-switched, CD38⁺ B_{mem} cells were found in spleen, medLN, and lungs of BALB/c mice (77). Furthermore, reactivation of lung B_{mem} populations was shown to contribute to enhanced immunity during virus challenge (77, 79) and the loss of B_{mem} cells, as seen in HIV-infected patients, reduced the patient’s ability to respond to influenza vaccinations (80). As outlined above, repeated exposure to influenza virus continuously alters the virus-specific class-switched B_{mem} compartment of humans, indicating that B_{mem} cells respond to and help control influenza infections, likely including many that cause subclinical infections.

B cells as regulators of T cell-dependent antiviral immunity

Several influenza virus infection studies in mice demonstrated B cell synergy with either CD4 or CD8 T cells in optimizing protection against a primary viral challenge. Although lack of CD8 T cells delayed clearance of influenza A/PR8 (H1N1) significantly in mice (81), so did a lack of B cells, albeit not as strongly (14). The targeting and elimination of DCs by cytotoxic CD8 T cells may also affect B cells directly by reducing priming of robust CD4 T cell responses that support development of virus-specific Abs. Consistent with that, the lack of CD8 T cells actually led to increased Ab responses (82). Absence of both CD8 T cells and B cells led to

uncontrolled viral dissemination and death (34), indicating CD4 T cells alone cannot protect against this infection. The same held true for CD8 T cells, as lack of both CD4 T cells and B cells resulted in significant mortality after low-dose H1N1 infection (83).

This raises the question of how B cells cooperate with T cells to prevent influenza virus-induced immunopathology and death. A lack of Ag-specific Ab in a *Listeria*-lymphocytic choriomeningitis virus challenge mouse model led to uncontrolled CD4 T cell activity, cytokine storm, and death, which was abrogated upon transfer of Ag-specific B cells (84). In that case, Ag-specific Ab was shown to reduce Ag load and thereby prevent continued overly strong activation of effector CD4 T cells. Consistent with that data, influenza-infected mice lacking B cells and chronically depleted of CD8 T cells, but with an intact CD4 T cell compartment, rapidly succumbed to infection, although replenishment with virus-specific B cells rescued these mice (34). The data indicate that generation of protective Abs provides a bridge between CD4-driven, innate, and Ag-directed responses.

Inclusive with this dynamic of suppressive feedback, B cells also promote CD4 T cell activity. Absence of B cells in influenza-infected mice was associated with reduced systemic CD4 T cell proliferation and reduced polarization of Th1 cells in the lungs, which resulted in decreased viral clearance (14). This might be due at least in part through the ability of B cells to produce numerous cytokines, as B cell-derived IFN- γ , IL-6, and TNF- α were shown to support Th1 responses by CD4 T cells (85–87). In addition, production of IL-6 by B cells was shown to promote the development and/or maintenance of CD4 follicular helper T cells and thereby the regulation of antiviral Ab responses (88).

Triggering of cytokine production by B cells might be induced through their expression of a wide array of pattern-recognition receptors, including TLRs, retinoic acid inducible gene I-like receptors, and NOD-like receptors (89–91). Specifically, the activation of TLR7 on murine B cells has been shown to induce IFN- α production during influenza, although not to EBV infection (92, 93). B cells also express inflammasome complexes (94) and thus can promote Caspase-1-mediated processing of the inflammatory cytokines IL-1, IL-18, and IL-33 into their active forms (95). IL-1 β is particularly interesting as IL-1 knockout mice showed increased morbidity and mortality following influenza infection, which correlated with decreases in amounts of serum and airway influenza-binding IgM (96).

Cytokine production by B cells may also modulate potential overshooting cytokine responses, the so-called “cytokine storm”, observed following infection with particularly pathogenic influenza viruses. IL-10 can suppress such overshooting responses (97), and IL-10 production by regulatory B cells (“Bregs”) and plasma cells has been shown to be effective in modulating a number of inflammatory conditions (reviewed in Ref. 98). Given the close interaction of B cells with recently activated CD4 T cells in the bridging channels of activated lymph nodes, further analysis of immunomodulation of antiviral T cell responses by cytokine-producing B cells may provide important mechanistic insight into the regulation of effective antiviral immunity to influenza.

Effective heterosubtypic immunity to influenza

Effective immunity to influenza virus requires the presence of T and B cells that respond to more than one substrain of

influenza, so called heterosubtypic immunity. Studies conducted following the 2009 pandemic showed that humans harbored influenza-specific memory CD4 T cells that were cross-reactive between seasonal and 2009 pandemic H1N1 strains (99), as well as cross-reactive B_{mem} cells. CD8 T cell immunity to influenza has long been known to target highly conserved internal proteins of influenza, particularly the nuclear protein, which can provide some heterosubtypic immune recognition (100).

The contribution of each adaptive immune compartment to heterosubtypic immunity was evaluated in mice, demonstrating a most potent role for T cell-dependent Ab responses. Genetic ablation of CD4 T cells in mice following a sublethal dose of a H3N2 virus infection resulted in significantly enhanced mortality to subsequent challenge with a lethal dose of H1N1 (33). The same studies with B cell-deficient mice resulted in even greater mortality, although depletion of CD8 T cells had no effect (33). This was despite existing cross-reactivity of H3N2-primed CD8 T cells. As expected, CD8 T cell-deficient mice generated protective levels of neutralizing serum Ab against the heterosubtypic virus, although influenza-specific Ab titers in CD4 T cell-deficient mice barely rose above that of their naive controls (33).

Recent studies have identified the highly conserved “stalk” domain of the trimeric hemagglutinin molecule as a target of broadly cross-reactive and indeed cross-protective Abs (reviewed elsewhere Ref. 2). A current challenge for exploiting this information for the better design of vaccines is the finding that these epitopes are neither immunodominant nor do they induce neutralizing Abs (i.e., Abs that directly prevent the binding of the virus to target cells). Their effectiveness instead relies on Fc-mediated effector functions by innate immune cells, including the enhancement of Ab-directed cell cytotoxicity (ADCC) facilitated by the binding of specific Ig isotypes to activating Fc receptors (101, 102). Indeed, broadly neutralizing mAbs with disrupted Fc receptor binding did not protect against lethal influenza infection in mice, whereas identical clones with full Fc receptor binding potential completely prevented infection-induced mortality (103, 104). Unfortunately, immunization of mice with Abs holding ADCC-associated epitopes led to increased alveolar damage and mortality after boost with intact virus (105), demonstrating the double-edged sword of Fc-mediated ADCC during influenza infection, especially as a potential vaccine target.

Given the limitations of these naturally induced broadly protective Abs, it appears likely that the observed naturally developing T cell-dependent, B cell-mediated heterosubtypic immunity is provided not only by such cross-reactive protective Abs but also by the ongoing reshaping of the overall influenza-specific B_{mem} compartment by seasonal influenza virus exposures. Such exposures constantly boost and shape an increasingly broad repertoire of B_{mem} cells, generating a range of Ab specificities that are both neutralizing and nonneutralizing. Together, they provide strong protection, if not always from virus-induced disease, from serious illness and death. When failure of protection is observed (i.e., in the very young as well as the elderly), the lack of a functional broad and robust repertoire of responding B cells, either because they are underdeveloped or no longer functional, is likely the main driver of this failure of immune protection.

It seems counterintuitive then that the presence of pre-existing, humoral memory to influenza virus has been shown to prevent the induction of strong neutralizing responses to a related but distinct influenza strain under certain conditions (106, 107), a phenomenon known as “original antigenic sin.” In those situations, the presence of a broad repertoire of pre-existing, influenza-specific B_{mem} cells might lead to the activation of nonprotective B cells over naïve, but potentially neutralizing B cells. Alternatively, the rapid capture of Ag may prevent strong immune response activation. Although animal models show clear evidence for such a phenomenon, the extent to which such scenario may contribute to the lack of protection in humans remains unclear.

Conclusions

B cells are the drivers of immunity to influenza virus infection during both primary and repeat infections. Their responses differ with the stages of the infection and provide the following: 1) pre-existing natural and Ag-induced Abs to blunt the initial infection; 2) rapid Ab responses generated in EF responses from pre-existing high-affinity B cell clones that contribute to the early control of virus infection; and 3) GC B cell responses that generate a broad repertoire of Abs and B_{mem} cells in preparation of the next exposure to this virus. The repeated exposures to influenza virus continuously shape the repertoire of influenza-specific B cells that can continue this cycle of responses. Naturally induced B cell responses, although not always successful in preventing influenza infection per se, are generally extremely robust, allowing humans to draw, if not win, the battle against this rapidly mutating and highly successful pathogen by surviving it.

Disclosures

The authors have no financial conflicts of interest.

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