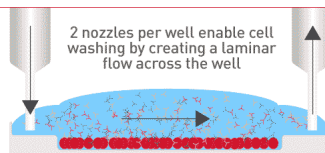


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Protective B Cell Responses to Flu—No Fluke!

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Protective B Cell Responses to Flu—No Flake!

Elizabeth E. Waffarn and Nicole Baumgarth

The mechanisms regulating the induction and maintenance of B lymphocytes have been delineated extensively in immunization studies using proteins and hapten-carrier systems. Increasing evidence suggests, however, that the regulation of B cell responses induced by infections is far more complex. In this study, we review the current understanding of B cell responses induced following infection with influenza virus, a small RNA virus that causes the flu. Notably, the rapidly induced, highly protective, and long-lived humoral response to this virus is contributed by multiple B cell subsets, each generating qualitatively distinct respiratory tract and systemic responses. Some B cell subsets provide extensive cross-protection against variants of the ever-mutating virus, and each is regulated by the quality and magnitude of infection-induced innate immune signals. Knowledge gained from the analysis of such highly protective humoral response might provide a blueprint for successful vaccines and vaccination approaches. *The Journal of Immunology*, 2011, 186: 3823–3829.

Simplicity sometimes has its advantages. Influenza virus has taken just such an approach to become a successful and notorious pathogen. Despite the small size of the virus' genome and the presence of only one gene devoted to immune evasion (NS-1, a type I IFN and caspase-1 blocker; reviewed in Ref. 1), respiratory tract infections with influenza virus cause worldwide between 250,000 and 500,000 deaths and affect 5–15% of the population each year (<http://www.who.int>). Failure of the mammalian host to generate long-term protective immunity against influenza is due to ongoing point mutations of the virus's surface receptors, hemagglutinin (HA) and neuraminidase (antigenic drift), and larger exchanges of entire gene segments (antigenic shift). Both processes enable the virus to evade neutralization by Abs but are too slow to result in evasion of clearance following infection of an individual; the virus is usually cleared within a few days. However, it does enable influenza to evade Ab-mediated immune protection at the population level, resulting in yearly waves of infections with newly emerging variants of previously circulating influenza virus strains.

These processes emphasize the effectiveness of Abs in preventing repeat infections with the same influenza strain and

also the shortcomings of the immune system in anticipating the virus' changing antigenic face. As a result, some have suggested that new vaccine approaches should be focused toward inducing CD8-mediated immunity, which is typically directed against more conserved, internal proteins of the virus (2). Given the potential for CD8 T cell-mediated tissue damage of the lung (3), and the fact that Abs together with innate signals are crucial for limiting initial viral loads to reduce the potential for T cell-mediated pathology, devising improved strategies for inducing potent and cross-protective Abs that prevent infection remains an important goal for combating this highly successful pathogen.

Unlike the subtle virulence tactics of influenza, there is nothing subtle about the B cell response to this infection. Each particular aspect of influenza infection is countered by a complex set of B cell responses that can prevent infection from occurring; when infections do occur, they can suppress early viral replication, help clear the infection, aid in tissue repair, and generate potent memory responses (Table I). In this paper, we review the current understanding of the induction and maintenance of the highly effective responses to this virus.

Quality and specificity of the influenza virus-specific humoral response
Homo- and heterosubtypic immunity. Following influenza infection, Abs are generated against most of the 10 viral proteins, although at greatly differing levels and kinetics (4). Best understood are the strong and often neutralizing responses against HA (5–7). Due to antigenic drift, and because current split-virus vaccines predominantly induce Abs to the mutating surface glycoproteins, the three influenza strains included in the yearly vaccine (influenza A/H1N1 and A/H3N1 and influenza B) are evaluated annually for their ability to generate neutralizing Abs to circulating seasonal influenza strains.

Vaccine or infection-induced homosubtypic (matching) neutralizing Ab responses are induced strongly in healthy individuals and contribute to virus clearance and also protect from repeat influenza virus infections (8). Mice cannot be reinfected with the same strain (9), even at doses 10,000-fold those used for primary infection (N. Baumgarth, unpublished observations). In contrast, B cell-deficient mice are vulnerable to reinfection (reviewed in Ref. 9), demonstrating the effectiveness of Abs in immune protection. Thus, lack of protection from the yearly flu in the human population is not due to defects in the antiviral B cell responses. Rather, the

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Abbreviations used in this article: AFC, Ab-forming cell; A/PR8, influenza virus A/Puerto Rico/8/34; DC, dendritic cell; HA, hemagglutinin; MHCI, MHC class II.

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Table I. Influenza infection characteristics and the B cell response

Influenza Infection Characteristics	B Cell Response Characteristics	References
Rapid infection; virus titers peak within ~72 h	Presence of protective, natural IgM Rapid development of strong extrafollicular foci responses	41, 42 4, 34
Localized infection; replication restricted to epithelial cells of the respiratory tract	Regional lymph nodes as main sites of B cell responses Formation of tertiary lymphoid tissues (BALT)	4, 27 26
Strong induction of local and systemic type I IFN	Strong and early Ab secretion in lung, and regional lymphoid tissues (IgG, IgA) Enhanced virus-specific Ab responses	4, 29–31 30, 67–70
Frequent exposures to homo- and heterosubtypic influenza virus	Enhanced lymph node size CD86-mediated enhanced Ab responses TLR7-regulated class-switch recombination Development of long-term AFC in bone marrow and lung Generation of circulating memory B cells	30, 68 72 75, 76 33, 39 5, 6, 40, 61

changing nature of the virus's antigenic structures renders Ab responses ineffective.

However, even nonmatching Abs may still prove beneficial; so-called heterosubtypic or cross-reactive and protective immunity, generated by previous encounters with a differing strain or substrain of influenza, has been linked conclusively to the presence of cross-reactive Abs (reviewed in Ref. 10), including broadly cross-reactive Abs to the 2009 pandemic H1N1 virus in humans (11). Broadly cross-reactive, neutralizing Abs to HA seem to bind predominantly, albeit not exclusively, to epitopes on the highly conserved helical region of the membrane-proximal stalk of HA1 and HA2 (11, 12), a promising potential target for new vaccine efforts (13). Apart from cross-reactive Abs to the influenza surface proteins, Abs to relatively conserved internal proteins of influenza can also provide heterosubtypic immunity and have become recent targets of new vaccine strategies. Immunization-induced Abs to the extracellular domain of matrix protein 2, induced only weakly after natural infection (14), can reduce disease symptoms in cotton rats (15). Nonneutralizing Abs to the influenza virus internal nuclear protein can reduce viral spread and mortality rates in mice (16, 17). Thus, pre-existing Ab-mediated heterosubtypic immunity to influenza can be beneficial, suggesting that even Ab responses to vaccines not perfectly matched to circulating strains might nonetheless reduce severity of disease.

Original antigenic sin. The above findings seem difficult to reconcile with the concept of original antigenic sin, the hypothesis that the presence of cross-reactive B cells to one influenza virus strain reduces B cell responses to a second encountered strain (18). This theory is based on experimental findings that the presence of Abs to a particular influenza virus strain can hinder viral clearance of a strain with differing HA and neuraminidase, suggesting that the presence of Abs to another influenza strain reduces a second Ab response. However, cross-reactive Abs do decrease viral loads compared with a completely naive animal, and an increased dose of Ag can overcome this response reduction (19), indicating that the magnitude of the Ab response adjusts according to viral loads. A recent intriguing study now suggests that more so than Ab levels, it is the avidity of Abs that is crucial for infection outcome (20). During the 2009 H1N1 pandemic, as in previous pandemics,

middle-aged subjects without pre-existing conditions and who had neutralizing Abs to seasonal influenza strains could become severely ill. These patients seem to have generated only low-avidity Abs to the pandemic strain, Abs that formed pathogenic lung immune complexes (20). Whether pre-existing B cell immunity to seasonal influenza strains contributed to or even caused the lack of high-avidity Ab generation remains an open question. The answer might ultimately determine the clinical relevance and/or context for the phenomenon of original antigenic sin.

Anatomical niches facilitate B cell responses to influenza virus infection

Influenza virus infection in humans and other mammals is typically a respiratory tract infection. B cells first encounter the virus, are primed, and differentiate to Ab-forming cells (AFC) within the respiratory tract. The highly tissue-specific nature of the B (and T) cell responses to influenza infection is a major obstacle in accurately assessing human adaptive immunity to the virus, because local responses are not always adequately represented by blood tests. This might explain the lack of clear correlates of protection seen with new, attenuated, intranasal live-virus vaccines, which provide immune protection, but often fail to induce significant serum Ab titers. In fact, Ab levels in the respiratory tract, but not the serum, best correlate with levels of protection from reinfection (21, 22). This hurdle might be overcome by measuring plasmablasts in peripheral blood by flow cytometry or ELISPOT analysis, a technology that seems to provide a highly sensitive way of assessing vaccine outcomes (8, 23). Such an approach has been successfully applied to detect IgA plasmablasts among PBLs, although the highly transient nature of their appearance requires accurate timing (23).

Tissue-specific factors likely shape the responses of B cells residing in and encountering Ag in the respiratory tract. Although this organ system is often referred to as a mucosal site, only the trachea and the larger bronchi possess a mucosa (24). Typical influenza infections begin in the upper respiratory tract and, if the virus is not eliminated, proceed toward the lung. Most studies with mice assess responses to pulmonary infections, as mice are resistant to upper respiratory tract infections, a limitation of the mouse as an animal model of disease. Whereas influenza virus replication and release of viral

progeny is observed only in respiratory tract epithelial cells, viral Ags are found also in various other cells in the lungs, including B cells (25).

Naive B cells are found in the organized lymph nodes draining the upper (cervical lymph nodes) and lower (mediastinal/bronchial lymph nodes) respiratory tract and interspersed in the lung interstitium (Fig. 1). Local inflammation, including infections with influenza virus, results in the formation of tertiary lymphoid structures, so-called BALT, along the branching points of the bronchial tree. BALT contains organized B cell areas, germinal centers, and AFC (reviewed in Ref. 26).

Ab production in the respiratory tract. The local draining lymph nodes act as major sites of B cell response induction following influenza infection (4, 27). B cells can encounter Ag through multiple routes. Dendritic cells (DC) in the lymph node medulla capture lymph-borne virus via SIGN-R1 for presentation to B cells (28). B cells might also directly capture and/or express viral Ags in the lymph nodes or at site of infection for transport to the lymph nodes (25). In both mice (4, 29, 30) and ferrets (31), the gold standard animal model, AFC are identified as early as 3 d after initial infection in the cervical and mediastinal lymph nodes as Ab titers rise in nasal washes and lung lavages. The extremely rapid kinetics of the infection-induced local B cell responses support a role for B cells in clearance of primary influenza infections (reviewed in Ref. 9).

Consistent with the nonmucosal nature of the lung, AFC in the lung generate mainly IgG and IgM, whereas 95% of Ab-producing cells in the upper respiratory tract are IgA (32). Despite the rapid kinetics of the lymph node B cell response, these lung AFC are not detected prior to day 7 in wild-type mice (33, 34) (K. Rothausler and N. Baumgarth, submitted for publication) and only after they are detected in the spleen

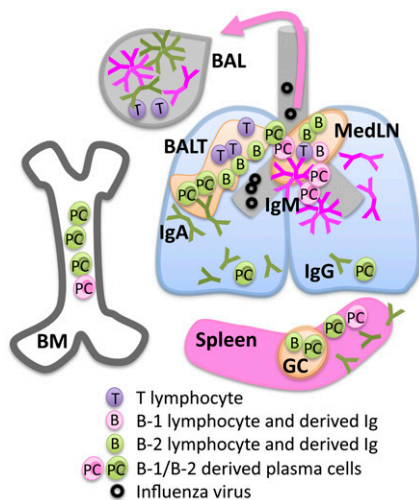


FIGURE 1. Anatomical distribution of B cell responses to influenza virus infection. B cell responses to influenza are induced mainly in mediastinal lymph nodes (MedLN) of the respiratory tract, where B-1 and B-2 cells differentiate to produce IgM, IgA, and IgG Abs within 3 d postinfection. Robust virus-specific Ab production in the lung airways is measurable immediately in the bronchoalveolar lavages (BAL) and with some delay in the serum. Seven to 10 d postinfection, AFC are found long-term in the lamina propria of the upper airways and in the BALTs. The spleen contains transient germinal centers (GC) and B-1 cell-derived natural Ab-secreting cells. Bone marrow (BM) AFC can be found beginning on day 14 and are maintained for life.

(33). Once induced, however, lung tissue AFC are present for life (33) (K. Rothausler and N. Baumgarth, submitted for publication). This raises questions about their tissue origin. Two possible pathways might generate lung-resident AFC: first, they might be induced in the draining lymphoid tissues and then migrate into the lung. Early migration studies showed that precursors of IgG-secreting cells from the mediastinal and bronchial lymph nodes preferentially home to salivary glands and the lung (35), suggesting that lymph nodes are the inductive sites of lung AFC and that priming confers tissue-homing specificity. Recent studies have implicated $\alpha 4\beta 1$ integrin (VLA4)–VCAM1 interactions in leukocyte migration to the lung (36). Once in the lung, B cells and/or AFC take residence within the lamina propria of the upper respiratory tract, lung tissue, and BALT. Production of BAFF and a proliferation-inducing ligand (APRIL) by DC might support long-term survival of plasmablasts/cells in BALT (37).

Alternatively, the BALT might facilitate both priming and maintenance of AFC in the lung tissue itself. The time needed for BALT formation in Ag-inexperienced mice would explain the delayed kinetics of AFC in the lung. Consistent with a role for BALT in AFC generation, germinal center-like structures and follicular DC are present within the BALT of influenza virus-infected mice (37, 38). Furthermore, interruption of BALT formation 2 wk postinfection reduces local IgA but not IgM production (37). However, it remains to be determined whether the initial induction and/or the maintenance of lung AFC require BALT. Overall, both secondary (regional lymph nodes) and tertiary (BALT) lymphoid tissues support B cell effector functions in the respiratory tract. A more complete understanding of their induction pathways could aid the development of vaccination strategies that mimic these highly protective Ab responses.

Systemic Ab production. Serum Ab titers, which are contributed by local and/or systemic AFC, are detected first around days 6/7 postinfection, delayed by at least 3 d compared with responses in the respiratory tract. They steadily increase for about 1 mo, and then relatively high Ab titers are maintained for life. Virus-specific AFC reside transiently in the spleen, starting around day 6/7 of infection, and long-term in the bone marrow (33, 39). In humans, oligoclonal populations of IgG⁺CD138⁺ AFC are present transiently in blood ~7 d following influenza immunization with live or inactivated influenza virus vaccines (8, 23).

IgG and IgA memory B cells to influenza are strongly induced and maintained for many months locally in the respiratory tract and systemically in most tissues (40). Thus, B cell induction following influenza infection occurs mainly in the respiratory tract, whereas effectors spread systemically: AFC to the bone marrow and memory B cells to just about every tissue of the body. It is unclear to what extent the systemic elaboration of Abs and distribution of memory B cells is proportional or even representative of the B cell response induced in the respiratory tract. This undefined relationship must be taken into consideration when measuring levels of Ab-mediated protection against influenza in human serum or plasma.

B cell response induction to influenza

Innate-like B cell responses. B cells contribute to protection from influenza virus infection even prior to any encounter with the

virus by generating natural IgM (i.e., protective Abs that are generated constitutively in the absence of antigenic challenge) (41–43). Influenza-binding natural Abs are produced almost exclusively by B-1 cells, a small subset of B cells characterized by unique developmental origins, phenotype, tissue distribution, and response regulation compared with conventional B cells (41, 44).

The many innate-like qualities of B-1 cells are highlighted by their responses to influenza. CD5⁺ B-1a cells, but not CD5⁻ B-1b cells, increase in frequency locally in the draining lymph nodes during acute influenza virus infection (days 5–10), and they secrete increased amounts of virus-binding IgM into the airways (29). However, influenza virus-binding IgM represents only a small fraction (~10%) of the overall increases in IgM production by B-1a cells in lymph nodes and airways, and the relative amounts of influenza-binding natural Abs do not increase over time compared with non-influenza-binding IgM. BrdU-labeling studies indicated a complete lack of B-1 cell clonal expansion over the course of the infection (29).

Thus, B-1 cells respond to influenza infection with redistribution to and differentiation at the site of infection while maintaining steady-state levels natural serum Ab levels (29, 41, 42). It is tempting to speculate that the redistribution of B-1 cells is a consequence of systemically elaborated innate cytokines. Consistent with this, influenza infection-induced type I IFN can profoundly affect leukocytes at distant sites (45). Furthermore, B-1 cells migrate from the body cavities to the gastrointestinal tract and spleen following injection of IL-5 and IL-10 (46), LPS, or bacteria (47). The latter was dependent at least in part on the adaptor molecule MyD88 (47), further indicating the innate nature of B-1 cell responses to this virus.

B-1 cells and secreted IgM might contribute to immune protection against influenza other than by virus neutralization (29, 42, 48). For example, B-1 cell-derived IgM is required for maximal induction of (B-2 cell-derived) influenza virus-specific IgG (42). B-1 cells are also known as strong producers of IL-10 (49) and thus could be involved in the regulation of local immune responses, similar to a recently identified regulatory role proposed for a B cell subset that shares some phenotypic characteristics with B-1 cells (50).

T-independent B cell responses to influenza. T-independent Ab production during influenza infection can provide a certain degree of protection, as mice lacking only CD4 and CD8 T cells survived infection longer than mice also lacking B cells (51, 52). Some studies showed that T-independent B cell responses could not facilitate viral clearance (52), whereas others found it sufficient for viral clearance and short-term protection (51). The disparate findings are due likely to differences in virus dose or virulence of the strains used for the studies. Early studies demonstrated the *in vitro* B cell mitogenic activity of influenza viruses carrying certain HA subtypes, possibly facilitated by their interaction with MHC class II (MHCII) I-E (53, 54). More recent studies suggested, however, that HA-induced mitogenic B cell activation requires signaling via MyD88, but not recognition of viral RNA (55), and thus could be due to engagement of TLR2 or TLR4 (20). The significance of these mitogenic effects on the overall B cell response to differing influenza strains is unclear.

T-dependent B cell responses to influenza infection. CD4 T cell deficiency results in a drastically reduced humoral response to influenza (52). Notably, although maximal antiviral IgG responses seemed to depend on CD4 T cells as well as B cell-expressed MHCII and CD40, maximal local virus-specific IgA required CD4 T cells but neither MHCII nor CD40 on B cells (27). The mechanisms underlying this CD4-dependent but cognate interaction-independent help for IgA production remain to be identified.

Early immunization studies with influenza virus A/Puerto Rico/8/34 (A/PR8) in BALB/c mice demonstrated that distinct waves of B cells, differing in their Ig repertoire, generate the overall strong antiviral Abs (5–7). The earliest-induced virus-specific Abs appeared relatively short-lived and could not be boosted, whereas Abs of late primary responses also contributed the secondary responses. These data can now be understood as the contributions of B cells with differing repertoires to the early extrafollicular and later germinal center responses, respectively. Indeed, HA-specific Abs encoded by one particular germline-encoded idiotype (C12Id), originally identified as contributing ~25% of the earliest Abs to A/PR8-HA (6), generate mainly extrafollicular foci postinfection (34). HA-specific C12Id⁺ cells generated germinal center B cells only infrequently (34) and, consistent with the observed lack of a C12Id contribution to a secondary response following immunization (6), did not give rise to memory B cells postinfection (K. Rothausler and N. Baumgarth, submitted for publication).

Such weak germinal center participation is not typical for influenza infection-induced B cell responses. Long-lasting germinal centers are found in lymph nodes and BALT for months following infection (34, 56). The C4Id encodes a prototypic response to A/PR8 HA during the later primary response in BALB/c mice (5). The lack of idiotype-specific reagents has prevented detailed analyses of this response at the cellular level. However, C4Id Abs carried frequent mutations in the CDR3 region during secondary stimulation, consistent with their germinal center origin (5). Thus, distinct populations of influenza virus-specific B cells exist that exhibit strong predilections for either T-dependent extrafollicular or germinal center responses.

What drives B cells toward either extra- or intrafollicular responses is unclear. Elegant studies using hen egg lysozyme BCR-transgenic mice recently suggested that high-affinity BCR–Ag interactions result in extrafollicular foci, whereas lower affinity interactions drive germinal centers (57). Not all studies are consistent with the affinity selection model, however, as some found stochastic selection of B cells into one versus the other response (58). Determining the signals underlying this selection event is of practical significance. The affinity selection model would predict that the overall Ab affinity to influenza in an individual might not increase over time with both extrafollicular and germinal center responses providing high-affinity Abs during different times postinfection. The ability to resist or to rapidly overcome influenza infection could then depend at least in part on the pre-existing B cell repertoire and the frequency of high-affinity B cells forming rapid, extrafollicular foci to influenza virus strains not previously experienced.

Systematic affinity measurements of early and later induced Ab responses to influenza in support of such a model are missing. However, infections of mice with VSV demonstrated

a lack of overall increases in Ab affinities over time due to the presence of high-affinity Abs early postinfection (59). Recent studies in humans also found that plasmablasts with relatively high affinity appeared in the blood within 7 d postinfection or vaccination with influenza (8). However, the latter data might be due either to the reactivation of memory B cells or be the result of a truly primary response.

Taken together, infection-induced extrafollicular foci provide rapid, highly selected, and protective Ab responses to influenza by mainly germline-encoded, high-affinity B cells. Germinal centers are long lasting in infected mice and result in the generation of AFC and memory B cells. Although the affinity selection model is consistent with the existing data on the B cell response to influenza infection, factors other than BCR affinity likely regulate the size of the extrafollicular response, as infections generate much larger extrafollicular responses than immunizations with influenza virus (J. Dieter and N. Baumgarth, unpublished observations).

Long-term maintenance of antiviral B cell responses. Specific IgG and IgA Ab production is maintained long-term after influenza virus infection of mice (33, 39) and humans. For example, up to 96% of people born between 1909 and 1919 in Finland had cross-protective Abs to the 2009 H1N1 pandemic strain, likely due to its relationship to the Spanish flu pandemic strain that circulated in the first part of the 20th century (60). These pre-existing cross-reactive Abs might be the reason for the unexpectedly low numbers of elderly adversely affected by the 2009 pandemic compared with seasonal influenza virus strains (reviewed in Ref. 20).

Long-term maintenance of such responses results from a combination of AFC and memory B cell induction. Bone marrow and lung microenvironments foster the long-term maintenance of AFC (33, 39), whereas memory B cells appear to distribute widely, with predilections for lungs, mediastinal lymph nodes, and the nasopharyngeal-associated lymphoreticular tissue environments (40, 61). Numbers of influenza-specific memory B cells in the lung far surmount those in the bone marrow (40). Whether the lung always harbors specific niches for these cells or whether they are infection-induced and how they are maintained long-term are important unresolved questions.

Regulation of antiviral B cell responses by innate signals

Complement. B cells participating in the response to influenza virus infection encounter a changing milieu of stimuli at the site of infection and in the draining lymph nodes that likely affect their ability to mount antiviral responses (Fig. 2). The importance of complement for the development of robust B cell responses has been demonstrated for numerous Ags, including influenza virus (reviewed in Ref. 62). It seems to affect all stages of B cell response induction, from supporting Ag uptake and presentation by APCs for induction of acute and memory B cell responses (28), mediation of direct B cell stimulation via costimulatory molecules CD21/35, and enhancement of Ab-mediated viral clearance. Vaccination studies demonstrated the strong adjuvant effects of C3d, which induced strongly protective Ab responses when coexpressed in a DNA vaccine with influenza HA (63).

Inflammasome-induced cytokine production. Influenza virus infection triggers intracytoplasmic pattern recognition receptors RIG-I and NOD2 in certain cell types postinfection, causing

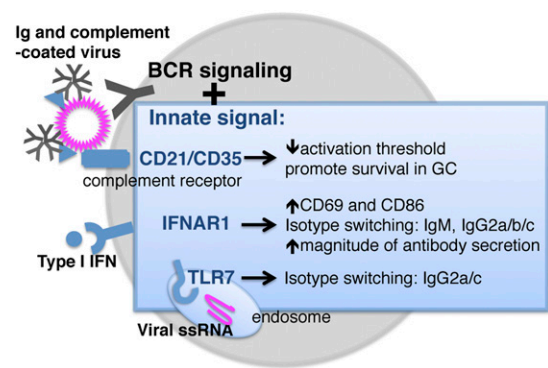


FIGURE 2. BCR-mediated and innate signals directly shape the B cell response to influenza infection. B cell responses to influenza are shaped by direct signals provided through the surface BCR and the type I IFNR (IFNAR1/2), as well as by engagement of complement receptors CD21/35. Following internalization of viral nucleic acid, TLR7 signals may alter the quality of the Ab responses. Mitogenic signals via TLR2/4 engagement by some subtypes of influenza HA might provide additional stimulation (not shown).

the induction of type I IFN, a strong antiviral cytokine and an immune modulator. Influenza also triggers inflammasome-mediated induction of IL-1 β and IL-18 through the engagement of RIG-I and possibly NLRP3 (reviewed in Ref. 1). The effects of NLRP3 or RIG-I-mediated caspase 1 activation and the subsequent release of IL-1 β and IL-18 on the B cell responses to influenza infection are incompletely resolved. One study concluded that induction of the inflammasome pathway is required for maximal production of antiviral serum IgM and IgG and intranasal IgA (64), whereas studies in IL-1R^{-/-} mice revealed a distinct deficit in influenza-specific IgM but not IgG or IgA secretion following influenza infection (65) (E.E. Waffarn and N. Baumgarth, unpublished observations). Others failed to find any evidence for effects of caspase-1 or cryopyrin, another component of the inflammasome complex, on B cell response outcome (66). Because the NS-1 protein of influenza virus can block caspase-1 activation (1), differences in the outcomes of these studies might be due to the use of distinct influenza strains or doses of infection. The studies did not distinguish direct from indirect effects of inflammasome activation on antiviral B cell responses; thus, the mechanisms by which Ab production could be affected are unknown.

In contrast, studies on the role of type I IFN have resulted in clear demonstrations of both direct (30, 67, 68) and indirect (69, 70) effects of type I IFN on B cells. Indirect effects include its enhancing effects on regional lymph node sizes postinfection (30, 67) and the development and size of germinal centers (N. Baumgarth, unpublished observations). IFN can also drive plasma cell generation via induction of IL-6 production by APC (70). Notably, type I IFN directly activates all regional lymph node B cells, but not those of the spleen or lung, as early as 24–48 h after influenza infection (30), resulting in rapid and extensive gene expression changes, including upregulation of surface CD69 and CD86 (67). Thus, the first signal regional lymph node B cells encounter following influenza virus infection is neither Ag nor T cell help, but type I IFN. Intrinsic changes affect both the quality and magnitude of the influenza-specific B cell response; mice with a B cell-specific deficiency in type I IFNR showed reductions in plasma cell numbers and in virus-specific IgM,

IgA, IgG2a/c, and IgG3 AFC, whereas IgG1 AFC were increased after influenza infection (30, 68). Upregulation of CD69 might cause a selective retention of B cells in regional lymph nodes (71), whereas enhanced CD86 expression might induce rapid Ab secretion by memory B cells (72).

TLR-mediated B cell response regulation. Another direct effect of type I IFN is the rapid but transient induction of TLR3 and TLR7 expression by regional lymph node B cells (67) (S.O. Priest and N. Baumgarth, unpublished observations). TLR7 is a major pattern recognition receptor for influenza, and its overexpression in B cells has been linked to autoimmunity (73). Studies on the effects of B cell-expressed TLR on B cell responses have shown conflicting results (74). TLR7, but not TLR3, appears to shape the Ab isotype profile of influenza-specific B cell responses. TLR7 plus type I IFN signaling can drive class-switch recombination to IgG2a/c, whereas its stimulation without type I IFN signaling drives IgG1 (75). This is consistent with studies showing that a lack of type I IFN direct signaling following influenza infection decreases IgG2a/c and increases IgG1 (30). Recently, TLR7 signaling was identified as the main mechanism for enhanced B (and T) cell responses after live virus infection compared with split virus vaccinations, although the target cells of the TLR signals were not identified (76). Given the extensive gene expression changes brought about by type I IFN signaling on B cells at the site of infection, it is likely that additional direct effects of innate signals guide the virus-induced B cell response.

Thus, innate signals elaborated during influenza infection modulate B cell responses to infection by acting both directly on the B cells and indirectly via signaling to DC and other cells. The relative lack of TLR7 and/or inflammasome signaling induced by the current split-virus vaccines may be responsible for the differences in effectiveness of vaccine- over live virus infection-induced responses.

Conclusions

Influenza infection triggers a robust B cell response in the lymphoid tissues of the respiratory tract that provides immune protection from both primary and secondary infections. The regulation of this B cell response highlights the complexities of humoral response induction and maintenance to respiratory tract pathogens. Multiple B cell subsets and waves of B cells with distinct Ig repertoires generate a multifaceted humoral response that provides protective Abs before, during, and postinfection both locally and systemically. Innate signals are important regulators of the antiviral B cell response, and future work is likely to identify additional innate signaling pathways that regulate this crucial arm of the immune system.

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Disclosures

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