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The role of innate signals in B cell immunity to influenza virus

Stephen O. Priest[1], Nicole Baumgarth[1]

[1]Graduate Group in Immunology and Center for Comparative Medicine, University of California, Davis, Davis, CA 95616, USA

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1. ABSTRACT

Decades of research on mammalian immunity to influenza virus infection have thoroughly established the important contributions made by both the innate and adaptive responses in containing the infection, and in eliminating the virus and protecting from reinfection, respectively. While rapid non-specific innate response is functionally distinct from, yet elegantly complementary to, the delayed-but-specific adaptive response, an increasing number of studies have provided evidence suggesting signals generated during the early innate response can have a significant impact on the quality of the later adaptive response, particularly in the context of influenza virus infection. From these findings emerged the notion that certain innate signals can act directly on B cells, and that this can even help activate virus specific B cells independent of T cell help, marking a major shift away from the current two-signal paradigm of lymphocyte activation. Here we review the current understanding of early B cell responses to influenza virus infection and the role of innate signals (particularly IFN-I and TLR7) in shaping this response.

2. INTRODUCTION

The importance of B cells in the immune response to influenza virus infection was first established by Iwasaki and Nozima, who found that mice depleted of B cells by chronic IgM-specific antiserum treatment, were unable to recover from the respiratory tract infection (1). While B cells were found to act synergistically with T cells in recovery from virus infection (2), studies using genetically B cell-deficient mu-MT mice depleted of helper T (Th) cells found that despite generating normal effector T cell responses, these mice were unable to control the infection. The significant contribution of naïve B cells in the absence of Th cells provided evidence that at least a portion of the recovery attributed to B cells is T cell-independent (3). Furthermore, natural antibodies provide further T-independent immune protection (4). Together, these studies define the essential contribution of B cells in the recovery from pathogenic primary influenza virus infection.

A major contribution of B cells for overcoming acute influenza virus infection can be attributed to their

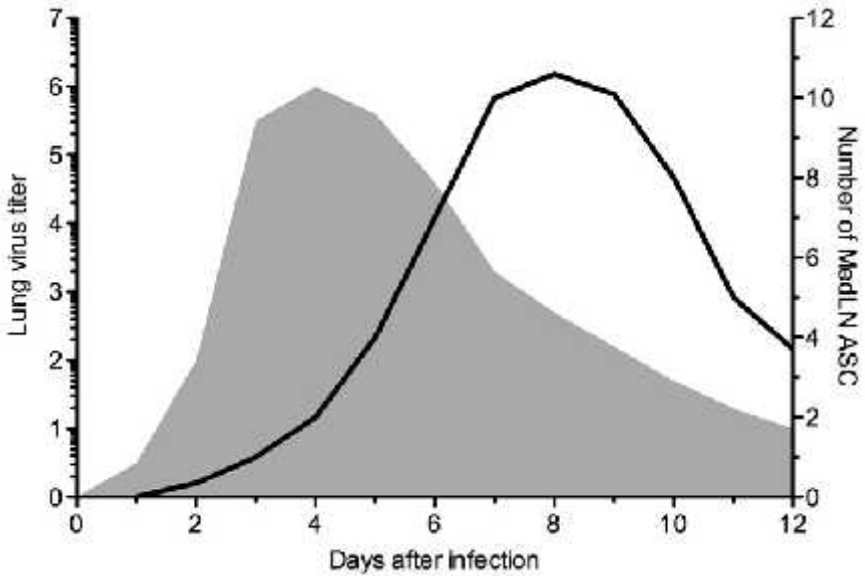

Figure 1. Kinetics of influenza virus infection and the local antibody response. Influenza virus replicates rapidly in the lung epithelium (grey filled curve), reaching peak titers within 3 to 4 days after infection. The lung virus load steadily declines thereafter, corresponding with the rising numbers of antibody secreting cells (ASC) in the mediastinal lymph nodes (black line). This response peaks between 7 and 10 days after infection, and begins to decline as the virus is cleared from the lungs.

ability to generate virus-neutralizing antibodies. Essential to controlling the infection is the activation of conventional B-2 cells and their subsequent production of virus-specific IgM and IgG (reviewed in (5). This activation generally involves two signals, one provided by the B cell receptor (BCR) binding to antigen, and the other via CD40-CD40L interaction by cognate T cells. However, the frequency of virus-specific B and T cells is low at the onset of infection. Therefore, a requirement for immediate B cell activation involving T cells seems difficult to reconcile with the rapid appearance of class-switched antibodies within the respiratory tract, seen as early as 48h after infection (6, 7). This, along with the studies mentioned above, implies that the B cells initiating the early virus-specific humoral response are activated via signals different from those provided by conventional T-dependent interaction. Recent studies suggest that innate signals, produced following the detection of virus infection, may fulfill this role.

This article will provide an overview of the B cell responses to influenza, and the innate signals triggered by influenza virus infection, which act directly on B cells to influence the anti-viral immune response. Among these signals, the anti-viral cytokine type-I interferon (IFN-I), and the virus-sensing Toll-like receptors (TLRs), have emerged as two key signals with distinct effects on humoral immunity. The convergence of these signals has been implicated also in triggering autoimmune disease in various animal models (reviewed in (8)). There are however, clear mechanisms through which these innate signals can regulate normal B cell responses to viral infections. A better understanding of these mechanisms could provide a more detailed picture of the regulation and disregulation of B cell activation by innate signals. Important for immune protection against influenza virus, this may support the exploitation of novel signaling pathways for enhancing the immunogenicity and efficacy of vaccines.

3. PRIMARY B CELL RESPONSES TO INFLUENZA VIRUS INFECTION

As discussed above, the B cell response to influenza virus infection generally begins with the binding of cognate antigen to the BCR, in conjunction with a second activating signal. This mainly takes place in the lymph nodes draining the upper and lower respiratory tracts, the cervical and mediastinal lymph nodes (MedLN) respectively, which are the major source of B cell responses during this infection (9). Antigen is thought to be transported from the lung tissue to these sites via the lymphatics, either carried from the respiratory mucosa by DCs or non-cognate B cells, or as particulate lymph-borne antigen that is captured by subcapsular macrophages (reviewed in (10)). In both cases, the antigen is transferred to follicular DCs present in the B cell follicle, which maintain the antigen in the lymph node for binding by virus-specific B cells (11). However, even prior to infection, considerable quantities of neutralizing so-called “natural IgM” is found in both serum and bronchiolar lavages of mice that provides some level of immune protection from primary infection with influenza (4, 12). The induction and regulation of these antibodies by B-1 cells is discussed below.

Within 3 days of infection, antibody-forming cells (AFC) can be detected in these local lymph nodes, corresponding with a rise in antibody titers in the respiratory mucosa and the peak of the lung influenza viral loads (6, 12, 13) (Figure 1). The rapid induction of antibodies following antigen or pathogen exposure is

produced by extrafollicular foci responses, induced by B cells of high-affinity for their cognate antigen (14) and generating Ig class-switched but short-lived plasma blasts (15-17). These strong early responses can contribute to immune protection from primary infections (18, 19). While extrafollicular foci can be both T-dependent (16) and T-independent (15), it appears that much of the early extrafollicular response to influenza infection is T-dependent (20). IgM production occurs first, preceding class-switch recombination and the subsequent production of IgA and IgG (9). The earliest response appears limited to the local lymph nodes. Antibody-forming cells (AFC) do not appear in the lung tissue before about 7 days after infection, after which time they slowly increase in frequency (20-22). This is around the same time that a small burst of AFCs in the spleen and antibodies in the serum are first detected. There has been considerable debate as to whether inducible lymphoid structures in the lung tissue, the bronchus-associate lymphoid tissues (BALT), are immune-inductive sites (23, 24). Recent studies by Randall and colleagues renewed this debate by demonstrating that BALT (they called it inducible, iBALT) in mice that lacked all other secondary lymphoid tissues could function to generate memory and its formation is enhanced on inflammatory stimuli (25, 26). The kinetics of the response seems not entirely consistent with such a notion; however, this requires further study (5). The location of B cell response induction is of importance, as it is likely that innate signals in lung, lymph nodes and spleen differ considerably following infection and thus could result in distinct B cell response qualities at these different locations.

Following increases of virus-specific antibody titers in the serum during the first month of infection, elevated antibody titers are observed in mice for life and are contributed, at least in part, by long-lived plasma cells in the bone marrow but not the spleen (27, 28). Long-term antibody-secretion has been observed also in the lung tissue (21, 27). Long-lived bone marrow plasma cells and memory B cell responses are thought to originate in germinal centers, in a strictly T-dependent manner, and are distinct from the early response generated by the short-lived extrafollicular foci (29, 30). The mechanisms underlying the induction and maintenance of long-lived B cell responses in the lung have not been fully elucidated.

Thus, primary B cell responses to influenza virus infection differ with regards to the time and location of their induction and maintenance. Given the highly localized nature of influenza virus infection, the virus only fully replicates in the respiratory tract epithelial cells, it is likely that B cell response induction in these different locations and times are affected differently by the elaboration of innate signals. Indeed, it is conceivable that the distinct B cell response outcomes and qualities might be regulated by the distinct environments; a possibility that is requires further exploration in the future.

4. INNATE SIGNAL-REGULATED B CELL RESPONSES TO INFLUENZA

4.1. Pattern recognition receptors triggered by influenza infection

As mentioned above, the tissue tropism of influenza A virus is generally limited to the epithelial cells lining the mammalian respiratory tract, which express specific receptors (sialic acids) and endonucleases that the virus requires for infection and production of infective progeny. These tissues support rapid replication of the virus, which reaches peak titers within 3 to 4 days (31, 32). The infected epithelial cells, along with alveolar macrophages and dendritic cells, detect the presence of the virus and begin producing innate cytokines and chemokines that effectively limit the reproduction and spread of the virus by inducing inflammation, and recruiting effector cells to the site (reviewed in (33)). This process of detecting and signaling the presence of infection is mediated by pattern recognition receptors (PRRs). These receptors are named for their ability to bind structured or repetitive patterns, known as pathogen associate molecular patterns (PAMPs), which are conserved among a broad range of pathogen species. PAMPs are typically derived from the unique biochemistry of microbes that are absent within the host, such as bacterial flagellin, cell wall components lipopolysaccharide (LPS), peptidoglycan and lipoproteins, and highly conserved microbial proteins, thus allowing PRRs to discern self from non-self (34). The viral envelope glycoprotein of influenza virus, hemagglutinin (HA), is one such PAMP known to exert a mitogenic effect in B cells (35). Alternatively, though not true PAMPs in this sense, most viruses are detected through their nucleic acids, either from the viral genome or produced as intermediates in the replication process. Three major families of pattern recognition receptors (PRRs) have been associated with innate responses to influenza virus infection: (i) nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs); (ii) retinoic acid-inducible gene-I (RIG-I)-like helicases (RLHs); and (iii) TLRs.

In infected cells, RLHs and NLRs expressed in the cytosol detect influenza virus RNA generated there during replication. The RLHs, specifically RIG-I, have recently been shown to initiate the inflammatory and antiviral response following recognition of single-stranded (ss) RNA from influenza A virus infection (36-38). Likewise, NLRs, specifically NLRP3 (NALP3/Cryopyrin/ NACHT–LRR–Pyrin (PYD)-containing protein 3), have also been implicated as important components for detecting RNA and initiating inflammasome/caspase-1 mediated IL-1beta/IL-18 inflammatory response to influenza (39, 40). These studies demonstrate the importance of RLH and NLR signaling in airway epithelial cells, but there is little data describing their expression and function in B cells, and it remains unclear what effect, if any, signaling through these receptors has on B cell function during viral infections. Given that B cells are not the target cells of influenza virus, nor have they been shown to support virus replication, the cytosolic localization of the RLRs and NLRs within these cells renders them unable to encounter their respective viral ligands (Figure 2). Signaling through these receptors is therefore unlikely to occur to a large enough extent within B cells to directly regulate their response to influenza virus infection. However, signaling initiated by these receptors in other cell types can lead to the production of other innate signals, such as type-I interferon discussed below, which can have a dramatic effect on the early humoral response to infection.

The best characterized of the PRRs are the TLRs. There is a substantial body of work describing their

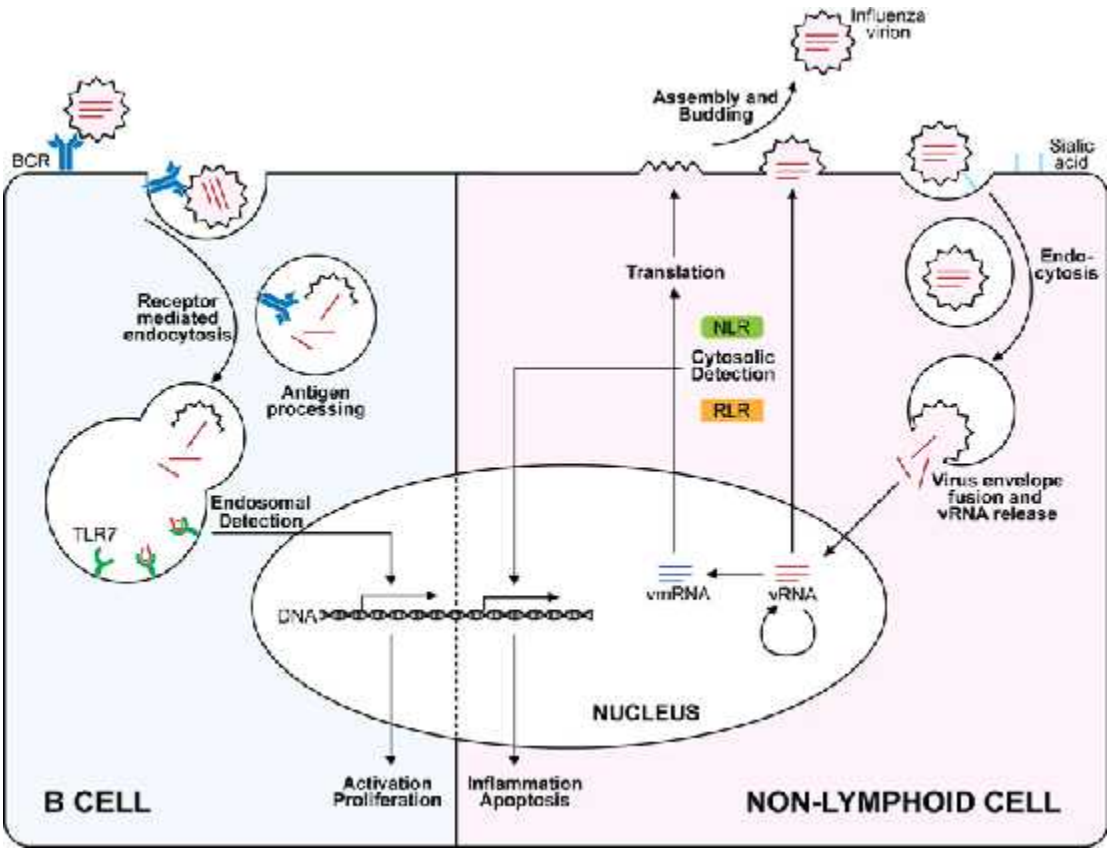

Figure 2. Mechanisms of influenza virus recognition in B cells and non-lymphoid cells. Influenza virus can enter the cytosol of non-lymphoid cells, specifically respiratory epithelial cells that express specific sialic acid residues exploited by the virus for entry into the cells. Following endocytosis, the viral envelope fuses with the endosomal membrane, releasing its genomic viral RNA(vRNA) into the cytosol where it can quickly enter the nucleus. Once in the nucleus, the vRNA is replicated and transcribed into messenger RNA (vmRNA). Both vRNA and vmRNA are then transported to the cytosol, where the vmRNA is translated into viral proteins that interact with the genomic vRNA at the cell surface to generate infectious progeny virions. The export of the viral RNA into the cytosol allows for recognition by RIG-I-like receptors (RLRs) or NOD-like receptors (NLRs), which in-turn induces transcription of genes responsible for inhibiting cell growth and initiating inflammation and apoptosis. Alternatively, B lymphocytes are not known to be infected by influenza virus, and therefore the cytosolic receptors are not likely to play a major role in recognition in these cells. Instead, the binding of whole virions or partial viral antigens to the B cell receptor (BCR) initiates endocytosis, bringing these antigens into the endosome where they can bind the intracellular Toll-like receptors (TLRs). Specifically, ssRNA from influenza virus binds TLR7, which rather than inducing an anti-growth state, leads to activation and proliferation of the B cell.

function in innate immunity, and their role in the development of the adaptive response. Seminal studies by the Flavell group elucidated dsRNA sensing TLR3 and ssRNA sensing TLR7 and TLR8, as essential components for eliciting innate and humoral immune responses to influenza virus infection (41, 42). These RNA binding receptors, along with dsDNA (specifically CpG DNA) sensing TLR9, are expressed within the endosomal compartments of cells, including B cells. This localization segregates these receptors from ubiquitous host-derived nucleic acids, as well as affords a degree of selectivity to what ligands these TLRs encounter. Unlike cytosolic receptor recognition in infected epithelial cells, B cells must actively uptake nucleic acid-containing antigens and transport them into the endosome in order for TLR recognition to take place (Figure 2). This can occur either by low level, fluid phase macropinocytosis, or by endocytosis following engagement of antigen with surface BCRs. During influenza virus infection, naïve B cells engage in this process, internalizing virus antigens generated in the interstitium of the lung and the draining mediastinal lymph node (MedLN). Thus, with steady-state expression of endosomal TLRs, and the ability to take up viral antigen, B cells at the site of influenza infection are in position to receive the earliest signals and initiate a rapid response.

4.2. TLR-mediated regulation of antiviral B cell responses

Current research has extensively detailed the role of TLRs in dendritic cell (DC) activation and function. More recent studies have addressed the impact of TLR direct signaling in B cells on antibody responses. Medzhitov and colleagues utilized mice whose B cells

lacked MyD88 adapter protein, an essential signaling component of all TLRs with the exception of TLR3. Following immunization with ovalbumin (OVA) in complete Freund's adjuvant (CFA), they found significantly diminished antigen-specific antibody responses to immunization with both T-dependent and T-independent antigens, despite the presence of fully activated DCs and T cells. Furthermore, in their hands TLR signaling in B cells was required for the production of IgM, IgG1 and IgG2c, but dispensable for IgG3 (43). This study seemed to support the concept that adjuvants stimulate adaptive immune responses by signaling through TLRs. In contrast, reports from the Nemazee and the Rawlings groups did not support these conclusions (44, 45). Gavin *et al.* found only a very limited effect on the levels of IgG2b and IgG2c induced following immunization with a T-dependent antigen (TNP-KLH) in one of four adjuvants tested (monophosphoryl-lipid A/trehalose dicorynomycolate ("Ribi")) in mice that lacked both adaptor molecules downstream of TLRs, namely MyD88 and TRIF. Furthermore, they saw no effect of TLR-signaling on T-independent responses (44). Furthermore, the modest effects of TLR-signaling on antibody-levels in non-intentionally exposed animals indicated that TLR signaling may control the class rather than the magnitude of immunoglobulin production in naive mice. A similar conclusion was reached by Meyer-Bahlburg and colleagues. They conducted immunization studies that showed only modest effects on the levels of IgM but not IgG responses to a T-dependent antigen in MyD88-/- mice (45). Thus, TLR-signaling appears to control the quality rather than the quantity of B cell responses. Recently, it was shown that the alum adjuvant activity is mediated by the release of host DNA from dying cells, which acts as a damage-associated molecular pattern (DAMP) independent of TLR9-signaling and independent of other known DNA-receptors (46). Interestingly, the same study found differential effects of IRF-3-dependent signaling of the host-DNA on class-switching to IgG1 and IgE, respectively (46). This further suggests that TLR-mediated and other innate signals might not drive B cell activation per se, but that these factors regulate and fine-tune the quality of B cell responses induced following vaccinations.

The role of TLR-signaling in the control of B cell responses to infections were studied by Marsland and colleagues using a mouse model influenza virus infection. They found that despite developing normal T-cell responses, mice lacking MyD88 had a diminished IgG2a/c response (47). Additionally, both MyD88- and TLR7-deficient mice developed an enhanced IgG1 response. These findings are consistent with the reports discussed above and implicate TLR signaling in the regulation of antibody class-switch recombination. The extent to which direct versus indirect effects of TLR signaling controls these B cell responses remains to be studied. However, the ability of B cells to produce virus-specific IgG, in the absence of T cell help (48, 49), suggests a likely direct effect of TLR-signaling or other innate signals on class switching during early B cell responses to pathogens. Collectively, these studies also point to qualitative differences in the B cell responses to peptide-antigen immunization and infections, respectively. A likely explanation for these differences is the presence of distinct innate signals such as DAMPs and PAMPs while B cell responses are formed. During influenza infection, B cells in the regional lymph nodes would be exposed to signals associated with both, the virus-induced tissue-destruction as well as early signals provided directly by viral antigens. This might lead to differences in the induction of co-stimulatory molecules on the surface of B cells, which in turn affect the proliferation, and production of virus-specific antibodies. This is supported by findings linking TLR7 stimulation with enhanced immunogenicity of whole inactivated virus vaccine when compared to split or subunit vaccines. (50)

4.3. Innate-like B cell regulation during influenza virus infection

Humans and mice generate systemic and mucosal "natural" antibodies in the absence of antigenic challenges (51-53) that are mostly IgM (systemic) or IgA (mucosal) and, due to their polyreactive nature, can bind numerous self- and foreign antigens, including pathogens (reviewed in (54)). We and others demonstrated a crucial role for these antibodies in protection from various bacterial and viral infections (4, 55-59). Most relevant here, mice that lack only natural IgM secretion showed significantly reduced survival from influenza virus challenge infections (4) (Figure 3). Both in humans and mice, these potent antibodies are produced by B-1 cells (CD5+ B-1a and CD5- B-1b); B cells that are distinct in development, phenotype and tissue location from conventional (B-2) B cells (4, 60-62). In contrast to the highly subtype-specific conventional antibody responses to influenza, natural antibodies bind to a wide array of influenza virus strains (4, 12, 57), thereby contributing to cross-reactive (heterosubtypic) immunity. In addition to its virus neutralizing ability, natural IgM is required for maximal induction of antiviral IgG responses to influenza and other pathogens (4, 55).

A memory-like characteristic of B-1 cells is the relative high frequency of cells that bind a particular antigen. About 10% of mouse B-1 cells bind to a range of influenza A and B viruses (12, 63). This high frequency is due likely to their polyreactivity and the heavy skewing of their Ig-repertoire (64-66). Following influenza virus infection B-1a, but not B-1b cells, accumulate in the regional lymph node and begin to secrete both virus-binding and non-binding IgM (12). This response was not virus-specific however, as frequencies of B-1a cells secreting virus-binding IgM remained constant at about 10% and this redistribution occurred without significant clonal expansion, indicating an innate-like response (12). Thus, B-1a cells might increase antibody production without altering their overall Ig-repertoire (i.e. lack clonal expansion). Such an innate-like response pattern of B-1a cells fits their overall physiology (60), including their inability to proliferate in response to BCR-crosslinking (67). The innate signals that regulate this redistribution of B-1a cells to the site of infection have not been identified. Preliminary evidence points to a distinct role of IFN-I (Waffarn E.E. and Baumgarth, unpubl.), a cytokine

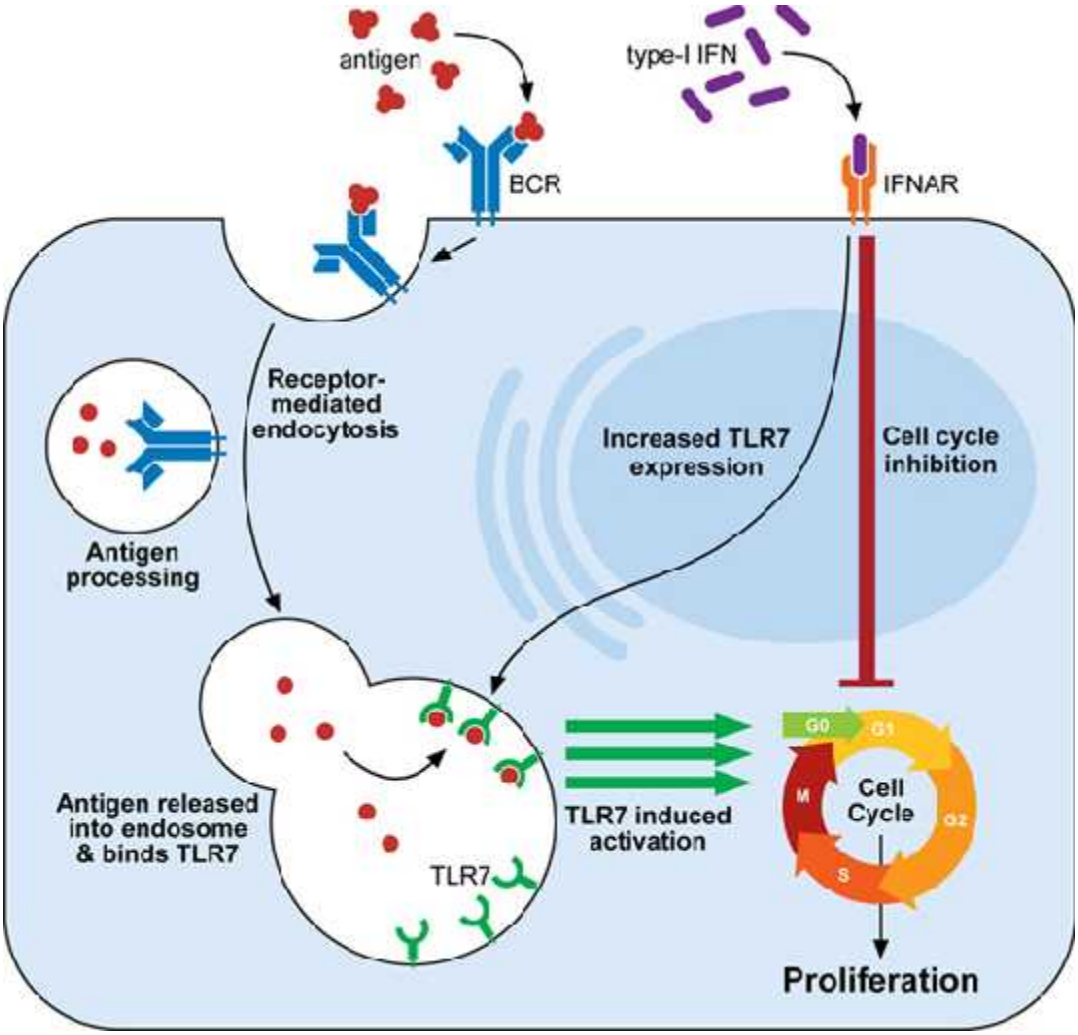

Figure 3. Opposing effects of type-I IFN in B cells enhances humoral response specificity. Type-I IFN produced by infected cells or pDCs during an influenza virus infection can bind the type-I IFN receptor (IFNAR) expressed on the surface of all B cells. This initiates signalling within the B cells that (A) induces the increased expression of TLR7 within the endosomal compartment, and (B) inhibits cell cycle entry. Because of the broad B cell expression of IFNAR, most B cells within the MedLN are stimulated by IFN-I during a virus infection. Therefore, the majority of the local B cells have simultaneously elevated and suppressed sensitivity to activation, via TLR7 expression and cell cycle inhibition respectively. Because TLR7 is expressed in the endosome, where it is sequestered from its respective ligands, most B cells won't be activated through this receptor. Thus the prevailing effect of IFN-I stimulation in most B cells will be suppression of activation, through inhibited cell cycling. A fraction of these B cells however, will be specific for a variety of viral antigens. These antigens can bind the surface expressed B cell receptor (BCR), which initiates receptor mediated endocytosis and uptake of the antigen into the endosomal compartment. Here the antigen is processed (degraded) as it is transported within the cell, finally fusing with another endosome where any virus derived ssRNA can be recognized by TLR7 and initiated activation and proliferation of the B cells. In these IFN-I stimulated virus-specific B cells, the increased expression of TLR7 effectively lowers the amount of RNA antigen necessary to initiate activation. The result is that the prevailing effect of IFN-I stimulation in these cells is the enhanced TLR7-mediated activation signal, which is able to overcome the IFN-I induced block in cell cycling and initiate proliferation. Meanwhile, this same block in cell cycling is prevalent in the surrounding population of IFN-I stimulated B cells that are not virus-specific, thus making them less sensitive to inadvertent non-specific bystander activation.

strongly induced by influenza infection (see below). IFN-I seems to stimulate B-1a cells in the pleural but not peritoneal cavity within as little as 24h after infection, altering their migratory behavior. Direct stimulation of B-1 cells by IFN-I appears required for maximal B-1a cell accumulation in lymph nodes after influenza infections, while it does not change their ability to secrete when in the lymph nodes (Waffarn E.E. and Baumgarth, unpubl.). This is consistent with reports by others, which demonstrated a role for MyD88-mediated B-1 cell stimulation following bacterial stimulation of peritoneal cavity B-1 cells (68-70). Thus, direct stimulation of B-1 cells via innate signals

shape this important innate-like B cell response during infections. Identifying the precise signals that control B-1 cell activation and migration might help the design of adjuvants that could induce such responses also during vaccinations.

4.4. Type I IFN-mediated regulation of antiviral B cell responses

4.4.1. Immune-enhancing effects of IFNa/b on B cell responses

When TLR binds its respective antigen, it initiates the MyD88 or TRIF adapter molecule-dependent signaling pathways, leading to downstream activation of activator protein 1 (AP-1) and nuclear-factor kB (NFkB) transcription factors, which initiate production of pro-inflammatory cytokines, including IL-1beta, IL-6, and TNF-alpha. Additionally, MyD88 and TRIF signaling pathways can activate interferon regulatory factor (IRF) 7 and IRF3 respectively, which induces the production of the key anti-viral cytokine family, IFN-I (34). Nearly all host cells are capable of producing IFN-I, however it is largely generated by infected lung epithelial cells and plasmacytoid DCs during influenza virus infection. Type I IFN comprises a family of at least 16 cytokines in humans and mice (13 IFN-alpha subtypes, IFN-beta, IFN-kappa and IFN-zeta, also called limitin), all of which utilize the same broadly expressed type I IFN receptor (IFNR) (71-73). These cytokines might shape humoral responses against influenza via direct and indirect mechanisms. Indirect regulation might entail IFN-mediated stimulation of myeloid DCs via induction of IL-6 production and thereby alterations in induction of T cell help. Also IFN-induced IL-6 production *in vitro* enhanced the differentiation of B cells to antibody-secreting plasma cells (74). Earlier, albeit somewhat contradictory literature also point to potent direct stimulatory and inhibitory effects of IFN on B cells (75-80). More recent *in vitro* studies showed enhanced anti-IgM-induced Ca-flux and B cell proliferation following recombinant IFN-stimulation (81). Others showed inhibitory effects of IFN-I on B cell proliferation (76). Much like TLR signaling, less is known about the role of B cell-intrinsic IFN-I signaling in the antibody response, though recent findings have drawn attention to its seemingly important contribution to adaptive response regulation.

IFN-I production is induced rapidly during influenza virus infection, corresponding closely with the increase in viral titers in the lung. During this early phase of the infection, IFN-beta predominates, produced mainly by the respiratory tract epithelial cells (33). B cells circulating through these local sites of infection are stimulated through type-I IFNR. These IFN-I-stimulated B cells can be seen mainly in the MedLN within 24 – 48h after infection at a time when cognate T cell stimulation is unlikely to occur given the low frequencies of virus-specific helper T cells, at least in an infection-naïve host at that time.

The rapid kinetics of IFN-mediated B cell stimulation highlight the presence of IFNR-mediated signals during the earliest B cell responses and their location-restricted effects. The outcome of IFN-I-mediated direct B cell stimulation appear multi-fold. Direct IFN-I-mediated B cell stimulation is clearly required for the induction of maximum antibody responses (6, 82), indicating a distinct T-independent, IFN-I-mediated effect on early B cell responses, although the precise mechanisms underlying this strongly direct immune-enhancing effect of IFN-I on B cells have not been fully elucidated. One potential mechanism is the induction of the costimulatory molecule CD86 by B cells. CD86-stimulation, even in the absence of BCR-mediated stimulation strongly induces antibody production by previously activated B cells *in vitro* and its expression by B cells is required for maximal antiviral antibody responses (83). Notably, both B cell-specific IFNR-/- and CD80/86-/- mice exhibited altered isotype profiles of their virus-specific IgG responses (6, 83). Similar findings were reported by Heer and colleagues in influenza infected TLR7- and MyD88-deficient mice, as well as in IFN-I deficient mice (47). Together, these findings establish type-I IFN as a direct innate signal in B cells that is essential in the initiation of the early antibody response against influenza virus infection.

4.4.2. IFNa/b regulates TLR-signaling by B cells

Recent reports have implicated the direct induction of TLR expression by IFN-I signaling in linking TLR-mediated and IFNR-mediated effects on B cell response regulation (84, 85). Likewise, we found that influenza virus infection induces TLR3 and TLR7 expression in an IFN-I-dependent manner (82). In light of a pivotal study showing that IFN-I directly augments the sensitivity of B cells to TLR7-induced activation (86), it appears that IFN-I might be regulating TLR7 mediated B cell responses by controlling expression of this receptor in B cells. Similarly, TLR9-mediated B cell activation was found to require priming by IFN-alpha (87). Our findings, however, are not easily reconciled with that latter study, as we find that *in vivo* or *in vitro* IFNR-signaling by B cells enhances their expression of TLR3 and TLR7 but not TLR9 and TLR4 (Priest, SO, Baumgarth N, unpubl.). The mechanism through which IFN-I augments TLR-mediated B cell activation thus is likely different between different TLR. This is supported by studies on B cell autoimmunity showing TLR7 signaling to exacerbate disease while TLR9 appears to play a regulatory role (reviewed in (88))

Studies implicating IFN-I and TLRs in the development of systemic lupus erythmatosis (SLE), an antibody mediated autoimmune disorder, provide some insights on the notion of IFN-I induced TLR7 expression and signaling. In a groundbreaking study, it was determined that the effects of the Y-linked autoimmune accelerator (Yaa) locus, known to induce SLE-like disease in the mouse model, was due to a duplication of the TLR7 gene on the Y-chromosome, leading to overexpression of TLR7. This was shown to lead to hyperreactive B cells that preferentially produce antibodies specific for RNA (89). This complements observations of elevated levels of IFN-I circulating in the serum of SLE patients (90), as well as cases in which patients undergoing IFN-I therapy develop SLE (91). These findings converge in the most recent data showing that direct IFN-I signaling in B cells can increase

expression of TLR7, and this subsequently enhances B cell activation through TLR7 engagement (92). These data all point to a clear role for IFN-I and TLR7 in the pathogenesis of SLE.

While these studies provide insight into innate regulatory activity in B cells, they address mechanisms of immune activation that are fundamentally different from those induced during influenza virus infection. In contrast to the sustained elevation of serum IFN-I in SLE patients, the induction of IFN-I, as well as the subsequent increase in TLR7 expression, is short-lived during influenza virus infection, and both return to normal within 4 days of infection (Priest, S.O. Baumgarth, N. unpubl). Such transient regulation co-localizes with the early production of virus specific antibodies in the respiratory tract; a phase of the anti-influenza response that is distinct from the later systemic production of antibodies (5). It is of obvious interest to determine if and how the induction of IFN-I and TLR7 during a virus infection might result in B cell hyperresponsiveness and autoimmunity, given the similar molecular signatures of B cells in these two very different disease states. From that it appears that additional levels of regulation are likely in place to constraint these innate signals during the normal antiviral B cell responses, thus avoiding the pathological consequences of non-specific B cell activation.

4.4.3. Immune-suppressive effects of type-I IFN on B cells

One of the best characterized antiviral function of IFN-I is its capacity for inhibiting cell cycling and proliferation (73). However, those studies have focused on its role in limiting viral replication in infected cell types, or in tumors as a potential therapy for cancer. Much of what is known about IFN-Is' anti-proliferative effects in B cells comes from studies in either B cell lymphomas or B cell lines (93-95). Though the cellular mechanisms of IFN-I mediated cell cycle regulation in these immortalized cells is likely to diverge from those of normal primary B cells, these studies can offer insights into potential pathways IFN-I might utilize in regulating B cell activity. One such study, using the Daudi B cell line, identified the cyclin dependent kinase inhibitor (CKI) p21(waf1/cip1) as an essential IFN-induced component regulating arrest, differentiation and apoptosis in these cells (94). Furthermore, this same inhibitor was shown in primary mouse splenic B cells to directly reduce proliferation, decrease responsiveness to nucleic acid antigen stimulation, and ameliorate disease in a mouse model of lupus (96).

In an equally striking study using monocyte-derived DCs, Hasan and colleagues identify a complex relationship in which IFN-I and TLR signaling control cell cycle arrest through antagonistic regulation of expression and degradation of the p27 CKI (97). Together, these findings provide evidence that IFN-I has negative effects on TLR-mediated B cell activation in addition to its stimulatory role described above.

Considering the studies described in this review, it seems likely that the combined effects of positive and negative signals on B cells cooperate to enhance the specificity of the early antibody cell response, while limiting B cell hyperactivation. These opposing effects might both be delivered by type-I IFN via divergent regulatory mechanisms (Figure 3): (1) the increased expression of TLR3 and/or TLR7 enhance the sensitivity of B cell recognition and activation in response to viral infection, and (2) the inhibition of B cell proliferation, potentially through changes in expression of select cell cycle regulatory factors. The endosomal localization of TLR3 and TLR7 limits their access to their respective ligands, which is vital to ensuring specificity of the B cell response. Most foreign antigens are internalized via BCR-mediated endocytosis following the cognate interaction of antigen with the BCR. The specificity required for this ensures that only viral antigens are internalized and detected by intracellular TLRs. This has been shown using LPS conjugate haptens, to which stimulated hapten-specific B cells considerably more than either ligand alone (98). By lowering the TLR signaling threshold required for B cell activation, IFN-I promotes the activation of those cognate B cells that can internalize the appropriate virus antigen. Meanwhile, the possibility of inappropriate bystander B cell activation is diminished by IFN-I's overall suppression of cell cycling. If correct this model would not only demonstrate the powerful positive and negative regulatory role of IFN-I in B cell responses, but it would also cast doubt on increased IFN-production as the main etiological origin of autoimmune diseases such as SLE.

5. CONCLUSION

Over the past decade, remarkable progress has been made in our understanding of the innate mechanisms of virus recognition and their role in the development of the overall immune response. With these advances came a novel appreciation of the distinct contribution made by direct innate signaling in B cells in shaping the adaptive response. This contribution involves the highly localized induction of type-I interferon early during influenza virus infection, priming MedLN B cells for subsequent activation by TLR signaling. These innate mechanisms ensure a rapid, virus-specific antibody response of a certain quality that is targeted to the site of infection. Though much of the currently published work addressing this activity has focused on the role of aberrant TLR expression or IFN-I signaling in the stimulation of self-reactive B cells and subsequent development of autoimmune disorders, this only reveals a portion of a complete picture. From the evidence described above, these signals have effects beyond their fundamental innate inflammatory and antiviral functions, and play some intrinsic role in regulating adaptive responses through direct B cell signaling.

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Abbreviations: type-I interferon, (IFN-I); dsRNA, double-stranded RNA; HA, hemagglutinin; LPS, lipopolysaccharides; NOD, nucleotide-binding oligomerization domain; NLRs, NOD-like receptors; PAMPs, pathogen-associated molecular pattern; PRRs, pattern recognition receptors; retinoic acid-inducible gene-I (RIG-I)-like helicases (RLHs), retinoic acid-inducible gene-I (RIG-I)-like helicases; ssRNA, single-stranded RNA; TLRs, Toll-like receptors

Key Words: Influenza, Virus, Infection, Respiratory tract, Hemaglutanin, HA, B-lymphocytes, B cells, Innate, Humoral, antibody, Immunoglobulin, Isotype, Ig, IgG, IgM, Toll-like receptor, MyD88, TLR7, TLR3, TLR9, Type-I interferon, IFN-alpha, IFN-beta, Cell cycle, Systemic lupus erythmatosis, SLE, Pathogen associated molecular pattern, PAMP, RIG-I, RIG-I like receptor, RLR, NOD-like receptor, NLR, CD80, CD86, CD69, Plasmacytoid dendritic cell, pDC, Mediastinal lymph node, Bronchus associated lymphoid tissue, BALT, Lung, Review

The role of innate signals in B cell immunity to influenza virus

Send correspondence to: Nicole Baumgarth, Center for Comparative Medicine, University of California, Davis, County Rd 98 & Hutchison Drive, Davis, CA 95616, USA, Tel: 530-754 5813, Fax: 530 752 7914, E-mail:nbaumgarth@ucdavis.edu