# B Cell Lineage Contributions to Antiviral Host Responses

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**Abstract** B cell responses are a major immune protective mechanism induced against a large variety of pathogens. Technical advances over the last decade, particularly in the isolation and characterization of B cell subsets by multicolor flow cytometry, have demonstrated the multifaceted nature of pathogen-induced B cell responses. In addition to participation by the major follicular B cell population, three B cell subsets are now recognized as key contributors to pathogen-induced host defenses: marginal zone (MZ) B cells, B-1a and B-1b cells. Each of these subsets seems to require unique activation signals and to react with distinct response patterns. Here we provide a brief review of the main developmental and functional features of these B cell subsets. Furthermore, we outline our current understanding of how each subset contributes to the humoral response to influenza virus infection and what regulates their differential responses. Understanding of the multilayered nature of the humoral responses to infectious agents and the complex innate immune signals that shape pathogen-specific humoral responses are likely at the heart of enhancing our ability to induce appropriate and long-lasting humoral responses for prophylaxis and therapy.

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**Abbreviations** BCR: B cell receptor; d.p.c: Days postconception; MZ: Marginal zone; TNP: Trinitrophenyl

## 1 Introduction

B cell responses are a major immune protective mechanism induced against a large variety of pathogens. A number of antibody effector mechanisms provide immune protection. For example, the direct binding of antibodies can interfere with pathogen attachment to host cells, thereby inhibiting cell entry and replication of intracellular pathogens. In addition, antibody binding can activate complement for direct destruction of pathogens, or interfere with other less well-understood mechanisms that inhibit infectivity of a pathogen.

The induction of B cell responses has been successfully exploited as the protective immune mechanism induced by most currently available vaccines, including inactivated influenza virus vaccines. Nonetheless, vaccine-induced responses are often of smaller magnitude and shorter duration compared to those induced to live-pathogen infection. While the underlying mechanisms for these differences are poorly understood, it is likely that the induction of innate regulatory mechanisms triggered specifically to certain classes of pathogens is at the base for some of those observed differences.

In addition to direct antibody-mediated pathogen neutralization, antibodies play a significant role in enabling or enhancing immune defenses. Importantly, many of these mechanisms have been associated with innate immune protection, which occurs very early after infection. Antibody-enhanced innate immune functions extend from activation of the classical pathway of complement by antibody–antigen complexes to antibody-dependent cellular cytotoxicity by natural killer cells. Table 1 provides a list of some protective mechanisms affected by the presence of antibodies. Given the low frequency of antigen-specific B cells at the onset of an infection and a general requirement for simultaneously induced T cell help, these findings raise the question of whether induction of early antibody responses underlies the same regulatory mechanisms as those induced later and those that form memory responses.

In this review, we will discuss the multifaceted nature of pathogen-induced B cell responses elaborated by the activation of distinct B cell subsets with unique functional characteristics and emphasize the role of B-1 cells in this process. The concur-

**Table 1** Antibody mediated immune protection during early infection

- Direct pathogen neutralization/inactivation
- Pathogen opsonization for uptake by macrophages and dendritic cells
- Natural killer cell activation and antibody-mediated cellular cytotoxicity
- Activation of the classical pathway of complement
- B cell activation via positive feedback stimulation through complement receptors
- Formation of antigen-antibody complexes for antigen-presentation by follicular dendritic cells

rent activation of various B cell subsets forms the basis for the large contribution of B cells in protection from both acute and recurrent infections. We will summarize our recent findings with regard to the antibody responses induced to live influenza virus infection and the simulation of bacterial infection via injection with LPS.

## 2 Multiple B Cell Subsets Contribute to Humoral Immune Defenses

Technical advances over the last decade, particularly in the isolation and characterization of B cell subsets by multicolor flow cytometry, have demonstrated the multifaceted nature of pathogen-induced B cell responses. It is now understood that in addition to the majority follicular B cell population, other B cell subsets contribute to many pathogen-specific humoral responses. Those additional B cell subsets are identified as marginal zone (MZ) B cells, B-1a and B-1b cells. Each B cell subset seems to require unique activation signals and to respond with distinct response patterns. Whether the follicular B cell population itself contains further subsets with differing activation requirements and/or functions is unknown. Current identification of B cell subsets is based mainly on cell surface phenotypic analysis, which identifies follicular B cells as a very homogeneous lymphocyte population.

### *2.1 Follicular B Cells*

#### 2.1.1 Development

Most B cells reside in the follicles of secondary lymphoid tissues of adult humans and rodents. Their developmental pathway from an uncommitted hematopoietic stem cell to an immature B cell in the bone marrow has been extensively characterized over the last 20 years (Hardy and Hayakawa 2001; Meffre et al. 2000; Rolink et al. 2001). The precise mechanisms that cause further differentiation or the death of immature B cells, once they have reached the spleen as so-called transitional B cells, are incompletely understood. Survival depends at minimum on the continuous presence of a BCR (Kraus et al. 2004) that lacks high-affinity/specificity to self-antigens (Goodnow et al. 1995). Follicular B cells have a half-life of 4–5 months in mice (Forster and Rajewsky 1990) and are continuously replenished from bone marrow precursors.

#### 2.1.2 Function

Follicular B cells, also termed conventional or B-2 cells, generate the bulk of the induced antibody responses following protein immunization. Responses are generated following establishment of vigorous germinal centers, seeded initially by follicular

B cells of relative low affinity for antigen (Paus et al. 2006). Much has been learned about the molecular mechanisms underlying conventional B cell response induction. In general, the responses are dependent on a minimum of two signals: B cell receptor (BCR) signaling via antigen binding and CD40–CD40L-mediated T cell help (Bernard et al. 2005; Noelle 1996). Thus, significant conventional B cell contributions require antigen-stimulated T cells, which are rare at the onset of an infection. These responses require some time to develop in order for clonal expansion of both T and B cells to occur and for T–B interaction to take place (Baumgarth 2000). Thus, T-dependent conventional B cell responses cannot be responsible for all rapid antibody responses generated to infections. Furthermore, recent literature suggests that innate third signals further enhance and modulate these responses. Two such signals have been identified to date as stimulation through TLR, via a MyD88-dependent pathway (Pasare and Medzhitov 2005; Ruprecht and Lanzavecchia 2006) and stimulation of B cells via type I (alpha and beta) interferon (Chang et al. 2007; Coro et al. 2006; Fink et al. 2006; Le Bon et al. 2006).

T-independent responses can be elaborated by follicular B cells and might explain in part rapid response induction to pathogens. Pathogens often express strongly structured, repetitive units on their surface. In the experimental setting, these are mimicked by haptens bound to a carrier backbone, creating multivalent binding sites (Bachmann and Zinkernagel 1997; see also the chapter by H.J. Hinton et al., this volume). Early studies on responses to the hapten trinitrophenyl (TNP) indicated that TNP-specific B cell responses to TNP-LPS are contributed by the same B cell precursors as those contributed by B cell responding in a T-dependent fashion (to TNP–red blood cell conjugates) (Lewis et al. 1978). Thus, TLR-mediated B cell stimulation, at a minimum stimulation through TLR4, might act to facilitate enhanced follicular B cell activation early during a response by providing directly and indirectly additional signals to B cells that can replace the need for T cell help. These third signals also support enhanced responses when T cell help is present (Pasare and Medzhitov 2005; Ruprecht and Lanzavecchia 2006).

## 2.2 *Marginal Zone B Cells*

### 2.2.1 Development

In the adult, MZ B cells and follicular B cells seem to develop from a common B cell precursor in the bone marrow. As their name suggests, following bone marrow development these B cells accumulate in the marginal zone of the spleen (Pillai et al. 2005). Establishment of the splenic marginal zone B cell compartment is slow, however, requiring some weeks to establish following birth or after whole body irradiation in experimental mouse models. In addition to these bone marrow-derived MZ B cells, there is evidence from studies in various genetically manipulated mice that MZ B cells are also contributed from precursors in fetal liver and spleen during the pre- and neonatal period (Carvalho et al. 2001; Hao and Rajewsky

2001; Heltemes-Harris et al. 2005). Ablation of de novo B cell development in adult mice via inducible deletion of the BCR causes a reduction of follicular B cells over time, while stable numbers of MZ or B-1 cells are maintained (Hao and Rajewsky 2001). Together, the data suggest that MZ B cells have significantly longer half-lives compared to follicular B cells and/or that their developmental origin is distinct from that of follicular B cells. Recent findings from a number of gene-targeted mice lacking various BCR-signaling components and transcription factors further indicate that MZ and follicular B cells require distinct signals for their development (Pillai et al. 2005). Importantly, exogenous antigen does not seem to be required for the selection of B cells into the marginal zone pool (Dammers and Kroese 2005). Phenotypically, MZ B cells are differentiated from follicular B cells by their high surface expression of the complement receptor CD21, CD9, low expression of IgD and lack of CD23.

### 2.2.2 Function

MZ B cells represent a B cell subpopulation uniquely positioned to provide rapid responses (Martin et al. 2001). Their location at the marginal zone of the spleen provides them with rapid exposure to blood-borne antigens. It also places them in close proximity to marginal zone macrophages that might present antigen and provide potential other innate stimuli. One consequence of splenectomy in humans and in experimental animal models is an increased risk of bacterial sepsis.

MZ B cells have a higher propensity to respond more rapidly and vigorously to innate signals such as LPS compared to follicular B cells (Martin et al. 2001). This is a reflection of their genetic make-up, which includes higher expression of various integrins and innate receptors compared to follicular B cells (Lopes-Carvalho et al. 2005). They also express higher levels of costimulatory molecules such as CD80/CD86 and in contrast to naïve follicular B cells are able to prime naïve T cells (Attanavanich and Kearney 2004). MZ B cell activation results in the establishment of vigorous extrafollicular foci responses, leading to strong early antibody secretion. While they can form germinal centers, those responses usually occur late in the response and are less prominent (Song and Cerny 2003). While MZ B cells might not generate vigorous germinal center responses, their early extrafollicular secretion of IgM does facilitate the establishment of germinal centers. The secretion of IgM by MZ B cells was shown to enable the complement receptor-dependent deposition of IgM-antigen complexes on the surface of follicular B cells (Ferguson et al. 2004). This is in agreement with studies in IgM secretion-deficient mice, which demonstrated the need for IgM in the establishment of strong IgG responses (Baumgarth et al. 2000b; Boes et al. 1998a).

The distinct tissue architecture of the spleen and the flow of blood that pools initially in the marginal zone sinuses make the spleen a unique and potent blood-filtering organ. Thus, splenic marginal zone B cells participate effectively in response to blood-borne and/or systemic infections. However, such infections are relatively rare, as the vast majority of infections occur via mucosal surfaces of the

gastrointestinal and respiratory tracts. Rapid local immune responses might thus not benefit from the presence of this cell population, since they occur primarily in the regional lymph nodes where afferent lymphatic enter the lymph nodes through the cortical sinuses. Lymph nodes lack phenotypic marginal zone B cell equivalents, i.e., CD21$^{hi}$ CD23$^{neg}$ IgM$^{hi}$ IgD$^{lo}$ cells. B cells in lymph nodes from noninfected or inflamed tissue sites appear very homogenous in flow cytometric analysis. Virtually all lymph node B cells are classical CD21$^{int.}$ CD23$^{+}$ IgM$^{lo}$ IgD$^{hi}$ follicular B cells. Ongoing studies suggest, however, that functional equivalents of splenic MZ B cells might exist (Rothaeusler and Baumgarth, see Sect. 3.2).

## 2.3 *B-1 Cells*

### 2.3.1 Development

The development of B-1a (CD5$^{+}$) and B-1b (CD5$^{-}$) cells is still incompletely understood and subject of some controversy. B-1 cells are the first B cells to develop in ontogeny (Kantor and Herzenberg 1993). The first (B-1 cell-restricted) B cell precursors are found in the splanchnopleure of the developing mouse embryo, approximately 7–9 days postconception (d.p.c.) (Godin et al. 1993). B-1 cells are also the main B cell population emerging from the fetal liver starting from around day 12 d.p.c. (Hardy and Hayakawa 1991).

Development of B-1 cells from dedicated bone marrow precursors after weaning is rare compared to B-2 cell development. While initial adoptive transfer studies suggested a complete absence of B-1 cell development from the bone marrow (Hardy and Hayakawa 1991; Hayakawa et al. 1985), our later studies demonstrated that adult bone marrow commonly reconstitutes a small population of B-1a cells and nearly half of the B-1b cells in irradiated adoptive recipients (Kantor and Herzenberg 1993; Kantor et al. 1995). More recently, studies with purified hematopoietic stem cells (Kikuchi and Kondo 2006) and B cell precursors (Montecino-Rodriguez et al. 2006) indicate that bone marrow contains a rare B-1 restricted precursor that can provide limited B-1 reconstitution. Findings from these latter studies (Montecino-Rodriguez et al. 2006) strongly support our long-held view that B-1 and B-2 cells belong to distinct developmental lineages (Herzenberg et al. 1986, 1992; Herzenberg and Tung 2006; Kantor and Herzenberg 1993).

Several studies suggest that B-1a and B-1b belong to separate developmental lineages (Herzenberg et al. 1992; Herzenberg and Tung 2006; Kantor and Herzenberg 1993; Stall et al. 1992). However, the question of whether B-1a progenitors, or B-1a cells themselves, can give rise to B-1b cells is still open. As in earlier adoptive transfer studies, we find that FACS-sorted CD5$^{+}$ B-1a cells commonly reconstitute a portion of the peritoneal (CD5$^{-}$) B-1b compartment (N. Baumgarth and L.A. Herzenberg, unpublished observations). Published studies by others also support the finding that transfer of FACS-purified CD5$^{+}$ B-1 cells can result in reconstitution of some B-1b cells (Haas et al. 2005). The interpretation of

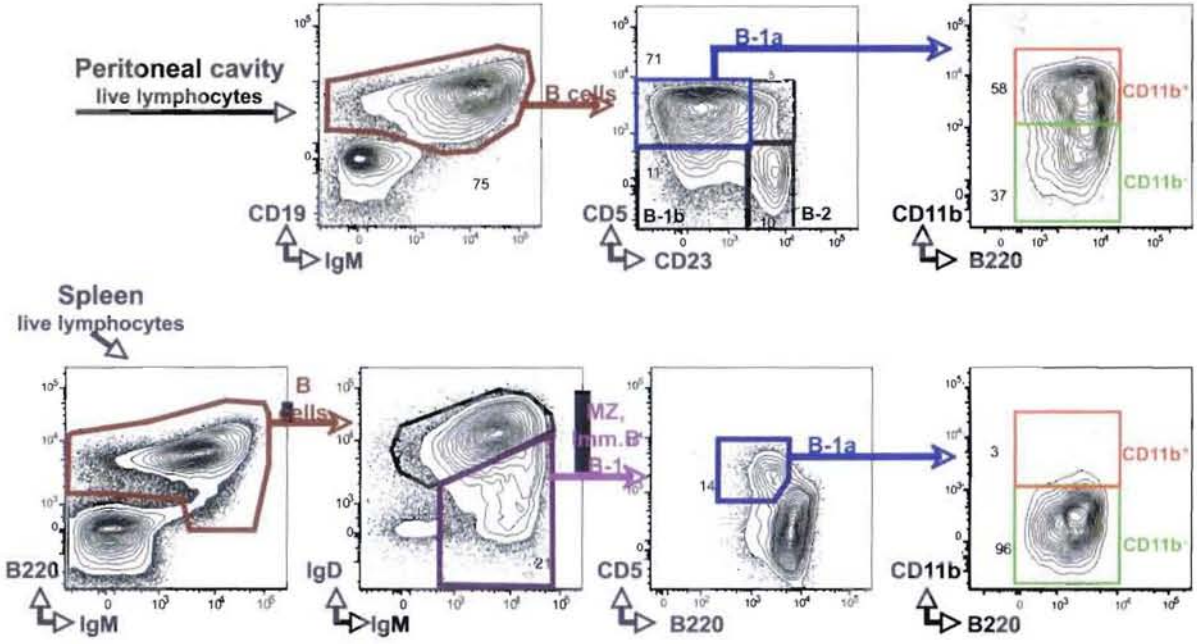


Fig. 1 Peritoneal cavity and splenic B-1a cells. Shown are 5% contour plot with outliers of peritoneal cavity (*top*) and splenic (*bottom*) cell suspensions. As shown, the majority of peritoneal cavity B-1a cells ($CD19^+$ $IgM^+$ $CD5^+$ $CD23^-$ B cells) express CD11b. In contrast, most splenic B-1a cells ($CD5^+B220^{lo}IgM^{hi}IgD^{lo}$) lack CD11b expression

B-1 cells are typically thought of as peritoneal and pleural B cells because they are the predominant B cell populations at these locations. However, it is important to recognize that despite their low frequency (~1%), substantial numbers of B-1 cells are found in the spleen. In fact, the total number of B-1 cells in the organ in unstimulated animals ($\sim 10^6$) is only slightly lower than the number of B-1 cells in PerC ($\sim 2\times10^6$). Furthermore, after LPS stimulation, the number of B-1 cells in the spleen parallels the increase in total cellularity, resulting in spleens that contain roughly $4\times10^6$ B-1 cells several days after stimulation, and hence a substantially larger number of B-1 cells than is typically found in PerC in unstimulated animals.

Consistent with the demonstration that B-1 cells disappear rapidly from the peritoneal cavity after intraperitoneal LPS stimulation (Ha et al. 2006), we have recently shown that the increase in B-1 cells after intravenous LPS stimulation is due to rapid migration and division of PerC B-1 cells into the spleen (Yang et al. 2007). These immigrants are identifiable for the first few days after they arrive in the spleen because they express CD11b at the same level as expressed on most PerC B-1 (Fig. 2). However, after 6 days, they lose CD11b expression and can only be tracked by experimentally introduced markers (e.g., Ig allotype). At least 30% of the immigrant B-1 cells in spleen divide within 1 day of LPS-stimulated animals. Within 2 days, roughly 10% of the cells that divided differentiate to become mature antibody-secreting plasma cells ($CD138^{hi}$ $Blimp\text{-}1^{hi}$ intracellular $IgM^+$). In contrast, minimal cell division and no detectable plasma cell development occur in PerC in the LPS-stimulated animals.

Importantly, there is an initial wave of plasma cell development that occurs in the absence of cell division (Yang et al. 2007). These plasma cells are all derived from resident B-1 cells that have terminated CD11b expression. Only a proportion of the resident B-1 cells participate in this initial wave, which enables the appearance of mature plasma cells 1–1.5 days after LPS stimulation (Fig. 2). The immigrant B-1 cells, in contrast, do not begin to reach the mature pool until 2 days after LPS. Thus, the first wave of innate antibodies produced in the spleen is selectively derived from a unique B-1 population that resides in the spleen and is capable of rapid differentiation in the absence of division.

Initial examination of the antibody repertoire expressed by the plasma cells that develop during the first wave of the response to LPS stimulation indicates that their repertoire is enriched for cells expressing common natural antibodies produced by B-1 cells (e.g., VH11Vk9, Y. Yang and L.A. Herzenberg, unpublished observations). In essence, the frequency of cells expressing this antibody is roughly three- to fourfold higher among these plasma cells than among the B-1 plasma cells that develop 2–3 days later. Thus, evolution appears to have devised a mechanism that places these well-known B-1 antibodies in a position to be the first to be produced when a bacterial stimulus such as LPS is encountered.

In addition to the important contributions of B-1 cells in providing protective natural antibodies to both bacterial and viral pathogens (Baumgarth et al. 2000a; Boes et al. 1998b; Ochsenbein et al. 1999), B-1 cells can also actively participate in the induction of at least some immune responses. Recent studies by Tedder's group suggest a division of labor by which B-1a cells provide natural antibodies

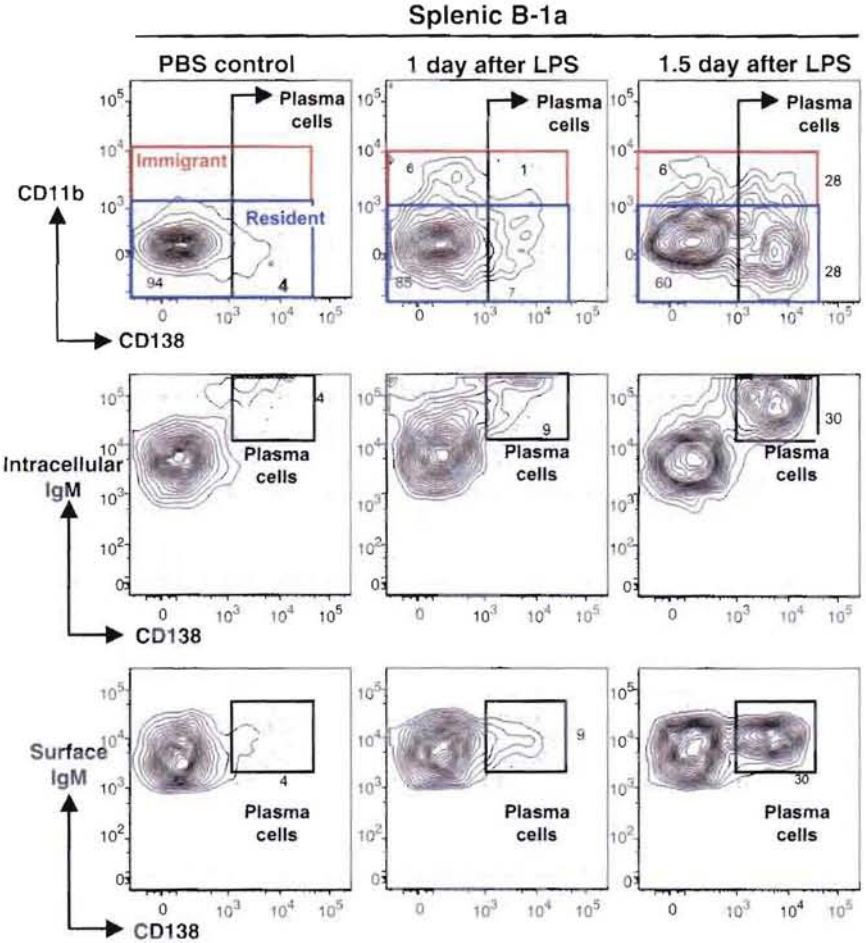


Fig. 2 Stimulation of peritoneal cavity B-1 cells with LPS triggers their migration to the spleen. Shown are 5% contour plots with outliers of splenic B cells gated as shown in Fig. 1. Cells were isolated from the spleen of mice injected with PBS (*left*) or LPS at 24 h (*middle*) and 36 h (*right panel*) prior to analysis. Note that new immigrants are distinct from resident B-1 cells by their expression of CD11b. Differentiation of resident B-1 cells to plasma cells is indicated by their upregulation of CD138 (syndecan-1) and high expression of intracellular IgM (*middle panel*), while maintaining surface IgM (*lower panel*)

and B-1b are induced to respond to *Streptococcus pneumoniae* infection with enhanced antibody secretion (Haas et al. 2005). This induced response seems to provide much of the polysaccharide-specific antibodies. Given the strong inhibitory activity of CD5 on BCR signaling (Bikah et al. 1996), these data provide an appealing explanation for the regulation of specific B-1 cell responses: B-1 cell responses would be restricted to those that do not express the inhibitory CD5 receptor.

In support of this view, studies with the relapsing fever-inducing spirochete *Borrelia hermsii* showed that protective activity could be transferred with IgM-secreting B-1b cells (Alugupalli et al. 2004; see also the chapter by K.R. Alugupalli, this volume) and that the B-1b cells in situ are sufficient for protection against the parasite (another example of B-1b cell responses). On the other hand, early work by Kenny and colleagues clearly established that B-1a cells ($CD5^+$) uniquely express T15-idiotype antibodies that react specifically with phosphorylcholine (Kenny et al. 1983; Knoops et al. 2004). IgM antibodies produced by these B cells are strongly increased in response to *S. pneumoniae* infection and contribute significantly to the primary response to the bacterium (Kenny et al. 1983). In addition, Peter Ernst and colleagues have shown that B-1a cells producing T15 idiotype antibodies are required for protection against mucosal (gut) infection (Pecquet et al. 1992a, 1992b). Thus, at least some B-1a cells contribute actively to protection against bacterial infection, raising doubt about the general applicability of the idea that B-1a play only a passive role, while B-1b cells play an active role immune responses (Kawikova et al. 2004; Pecquet et al. 1992a; Szczepanik et al. 2003).

## 3 Immunity to Influenza Virus Infection

Influenza's main evasion strategy relies on rapid replication and aerosol-mediated viral spread. Indeed, the relatively small influenza virus genome seems to contain only one gene (NS-1) involved in immune evasion strategies. NS-1 acts via binding to viral genomic single-stranded RNA, thereby inhibiting activation of the RNA helicase enzymes retinoic acid-inducible gene I (RIG-1) and the induction of type I interferon (Pichlmair et al. 2006). Infection with influenza virus induces an array of cellular and humoral immune defense mechanisms, both innate (Fujisawa et al. 1987; Reading et al. 1997) and adoptive (Doherty 2000; Doherty et al. 1997; Gerhard et al. 1997), which act in concert to provide strong protective immunity. Natural killer cells, macrophages (Fujisawa et al. 1987), natural antibodies (Baumgarth et al. 2000a), and the induction of type I interferon (Basler et al. 2001; Garcia-Sastre et al. 1998; Talon et al. 2000; Wang et al. 2000) and IL-1 (Schmitz et al. 2005) provide a first line of immune defense. Strongly cytolytic, virus-specific $CD8^+$ T cells (Doherty et al. 1997; Flynn et al. 1998; Hogan et al. 2001) and neutralizing antibodies (Gerhard et al. 1997) provide an effective way for removing virus-infected host cells and inactivating infectious virus. The development of humoral responses is of great importance, as they can provide disease-preventing sterile immunity through local production of antibodies (Renegar and Small 1991a, 1991b) and their induction through vaccination is currently used as an effective means of protection (Bridges et al. 2001).

The humoral response to influenza virus is comprised of different sets of antibodies: (a) natural antibodies, produced prior to any encounter with the virus (Baumgarth et al. 1999, 2000b). (b) virus-induced antibodies produced in

a T cell-dependent manner, and (c) virus-induced antibodies produced independent of cognate T cell help (Gerhard et al. 1997; Mozdzanowska et al. 1997, 2000; Sha and Compans 2000; Virelizier et al. 1974). These latter types of antibodies provide a strong component of the immune response. As we have shown previously, natural antibodies that bind influenza virus are crucial for survival from infection (Baumgarth et al. 2000a). Gerhard and colleagues showed that T-independent virus-induced B cell responses provide immune protection against influenza virus (Mozdzanowska et al. 2000). Thus, both natural antibodies, produced prior to encounter with pathogens and T cell-independent pathogen-induced antibodies are being increasingly recognized as important components of the humoral response. Understanding the contribution of distinct B cell subsets might help to determine the mechanisms of these unconventional responses.

## 3.1 *Follicular B Cell Responses in Influenza Virus Infection*

Follicular B cells generate the majority of the influenza-virus induced antibody responses. Much of this response is T-dependent, shown by the massive reduction in antibody levels in T-deficient or CD4 T cell-deficient or T-deficient nude mice. Following experimental infection of mice, germinal center responses develop in the draining mediastinal lymph nodes around day 6–7 of infection (N. Baumgarth and K. Rothaeusler, unpublished observations). At this time, systemic antibody levels in the serum are also detectable for the first time (Baumgarth et al. 1999; Baumgarth and Kelso 1996).

The local induction of virus-specific conventional B-2 cell responses in the draining lymph nodes seem uniquely affected by the presence of inflammatory cytokines. Studies on IL-1R1 gene-targeted mice showed an enhancing effect of virus-induced IL-1 production on IgM responses (Schmitz et al. 2005). Our recent studies demonstrated the type I interferon-dependent activation of all lymph node B cells early during influenza virus infection (Chen et al. 2007; Coro et al. 2006). This innate early B cell activation was not only required for strong antibody responses to the virus (Coro et al. 2006), it also had significant consequences for the response outcome following BCR-mediated stimulation (Chen et al. 2007). Interestingly, among the strongest infection-induced gene expression changes in regional B cells were the upregulation of TLR3 and TLR7 (Chen et al. 2007). TLR7 and type I IFN are important regulators of the isotype profile of the developing antiviral response to influenza (Coro et al. 2006; Heer et al. 2007). Thus, infection-mediated innate B cell stimulation alters the way in which B cells respond to both antigen and innate signals. How both specific BCR-mediated and nonspecific TLR-mediated signals are synthesized to optimize B cell response outcomes is an important question requiring further study. B cell response model systems in which B cell responses have been studied and which have formed the basis for our understanding of B cell

response regulation do not provide these innate signals and thus might not be sufficient to fully comprehend B cell response regulation to pathogen encounter. In addition, tissue-specific signals provided at the site of pathogen entry, most frequently the mucosal surfaces of the gastrointestinal or respiratory tract, might provide further signals distinctly regulating local compared to (splenic) systemic responses.

## 3.2 *MZ B Cells and Influenza Virus Infection*

Typical sublethal infection of mice (and humans) with influenza virus causes only localized respiratory tract infections, since the virus can only fully replicate in the respiratory tract epithelium. Therefore, the majority of viral antigen is not likely to enter the blood in any significant amount and marginal zone B cell responses would not be expected. However, ELISPOT analyses of murine spleen cells following influenza virus infection show a small but significant early and transient induction of virus-specific responses in the spleen (N. Baumgarth and K. Rothaeusler, unpublished observations). Flow cytometric analysis of spleens from influenza virus-infected mice demonstrates, however, a transient relative and absolute reduction in marginal zone B cells (Fig. 3). This latter finding is surprising, given that marginal

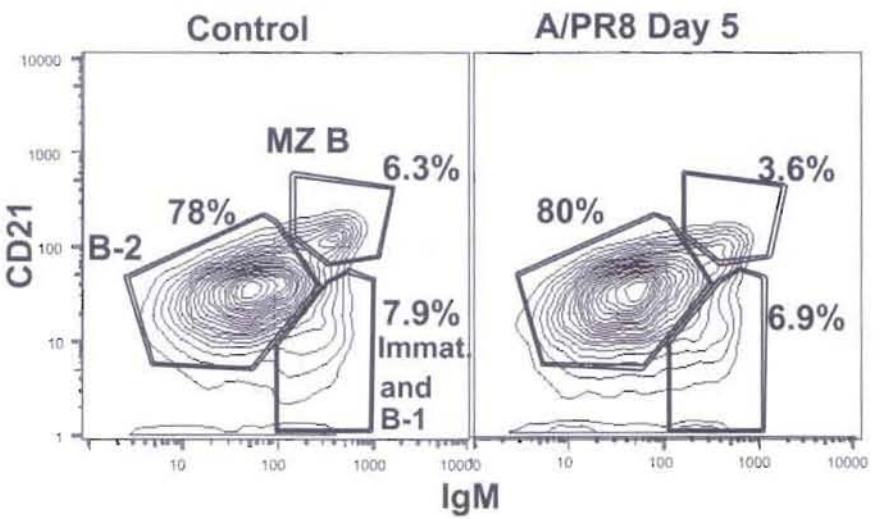


**Fig. 3** Reduction in marginal zone B cells following acute influenza virus infection. Shown are 5% contour plots from splenic cells selected for expression of the pan-B cell marker B220 and lack of expression of CD3, 4.8, F4/80 and exclusion of propidium iodide as live/dead discriminator. Numbers indicate the relative proportion of marginal zone B cells (MZ), follicular (B-2), and immature/B-1 cells in the spleens of noninfected (*left panel*) and 5-day influenza A/PR8-infected mice (*right panel*). Note the strong reduction in MZ B cells expressing high levels of CD21 and IgM

zone B cells are regarded as sessile. While we have not identified the mechanism for this temporal depletion of the marginal zone B cell population, it indicates that marginal zone B cells can react to tissue-localized infections, possibly in response to circulating cytokines or chemokines. Given the need for sphingosine 1-phosphate receptor 1 expression in the appropriate localization of MZ B cells (Cinamon et al. 2004), infection-induced alterations in expression of this receptor or its ligand provides an attractive possible mechanism.

Could these data also indicate a mobilization of marginal zone B cells to the site of infection? As stated above, $CD21^{hi}$ MZ B cells are not present in the lymph nodes prior to infection and we did not find any $CD21^{hi}$ B cells in the regional mediastinal lymph nodes at any time after infection (K. Rothaeulser and N. Baumgarth, unpublished observations). While it is possible that phenotypically altered marginal zone B cells might accumulate in the lymph nodes following infection, existing data do not support this conclusion. MZ B cells do not alter their cell surface phenotype when they are dislodged from their proper location by genetic ablation of S1P1 or treatment with FTY720, at least within the spleen (Cinamon et al. 2004). A more likely explanation is the migration of activated marginal zone B cells into the red pulp, where they might reside short-term as differentiated plasma cells.

Given the fact that most infections occur localized and are contained within a certain tissue, it appears counterintuitive that populations of rapidly responding B cells are found only in the spleen but not in tissue-draining lymph nodes. In addition, we have provided evidence that following influenza virus infection isotype-switched antibody responses are measurable as early as day 2–3 after infection locally in the lymph nodes (Coro et al. 2006). Because of these very rapid kinetics, it appears safe to assume that these cells were activated by mechanisms other than cognate T cell help. Interestingly, earlier studies by Gerhard and colleagues demonstrated a strong difference in the idiotypic profile of the anti-influenza virus responses following immunization with influenza A/PR8. In particular, those studies identified a germline-encoded immunoglobulin-idiotype (C12Id) that contributed up to 25% of the entire early anti-hemagglutinin response but was absent from later primary and a secondary response (Kavaler et al. 1990, 1991). Our preliminary studies indicate that this response also dominates early B cell responses to infectious influenza virus. Furthermore, they suggest that the C12Id-response shows many of the functional hallmarks of a marginal zone B cell response, but it is contributed by cells that identify as follicular B cells (K. Rothaeusler and N. Baumgarth, unpublished observations). Thus, these data indicate that in lymph nodes rapidly responding (follicular) B cells exist that fulfill at least some of the roles MZ B cells play in the spleen.

## 3.3 *B-1 Cell Contributions to Influenza Virus-Specific Immunity*

The lack of unique markers to identify B-1 cells has hampered our understanding of their contributions to immunity against infections. In order to study the potential role of B-1 cells in influenza virus infection, we have therefore utilized protocols to

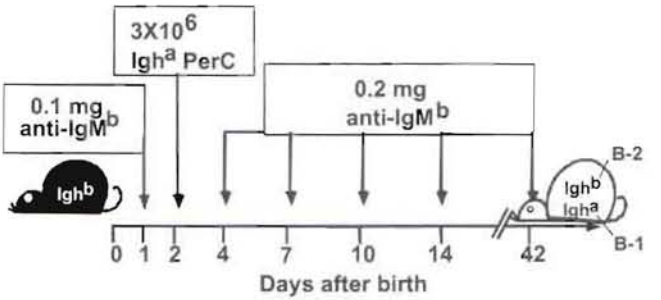


Fig. 4 Generation of B-1/B-2 allotype chimeric mice to track B-1 cell responses. Treatment of newborn mice with allotype-specific anti-IgM ablates all host-derived peripheral B cell development. This treatment is continued for 6 weeks. In this time, transferred congenic but allotype-disparate B-1 cells [or peritoneal cavity washout (PerC) cells as source of B-1 cells] expand. Four to 6 weeks following cessation of anti-IgM treatment, the host-derived B-2, but not the B-1 cell compartment is fully reconstituted. Donor-derived B-1 cells contribute >80% of the B-1 cell compartment of the mouse. (Baumgarth et al. 1999; Lalor et al. 1989a, 1989b)

track B-1 cells and their antibodies via immunoglobulin-allotype-specific markers (Fig. 4; Lalor et al. 1989a, 1989b). These mice are generated by treating newborn mice with host-allotype-specific anti-IgM on day 1 after birth and transferring congenic allotype-mismatched B-1 cells or peritoneal-cavity B cells on day 2 after birth. Four to six weeks after cessation of the anti-IgM treatment, host B-2 cells (but not host-B-1 cells) have returned to normal numbers. Most (>80%) B-1 cells in these mice are of the donor allotype, thus allowing tracking of donor-derived B-1 cells by their disparate Ig-allotype. While there are limitations to this approach, it provides a means of following B-1 cell responses without having to rely on potentially changing surface phenotypes.

### 3.3.1 Passive Contributions of Protection by Provision of Systemic Natural Antibodies

Using these allotype-chimeric mice, we demonstrated that mice harbor significant titers of natural, preinfection influenza-binding antibodies. These antibodies were generated mostly, if not exclusively, by B-1 cells (Baumgarth et al. 1999). Analysis of the serum antibody response to influenza virus further established that following infection the entire virus-induced antibody response, including virus-specific IgM, is derived from the host, i.e., the B-2 cell compartment. Thus, while B-1 cells seemed to generate virtually the entire natural antibody levels, they did not contribute to the systemic antibody response. Importantly, we showed that passive transfer of natural antibodies is at least partially protective and that the lack of IgM secretion by B-1 cells reduces survival from high-dose influenza virus infection (Baumgarth et al. 2000b). Thus, the generation of natural IgM importantly contributes to survival

from an acute viral infection. Infections with other bacterial and viral pathogens have similarly shown the importance of this evolutionary conserved antibody-mediated immune defense mechanism (Boes et al. 1998b; Ochsenbein et al. 1999). Whether these antibodies were elaborated by B-1a or B-1b cells was not delineated in those studies.

It is important to note that B-1 cells contribute significantly to mucosal IgA production. Roughly one-third of plasma cells in lamina propria of B-1/B-2 allotype-chimeric mice are derived from B-1 cells (Kroese et al. 1989). We have made similar observations for the respiratory tract (N. Baumgarth and L.A. Herzenberg, unpublished observations). This is also in good agreement with recent studies by Stavnezer and colleagues showing that B-1 cells preferentially switch to IgA in vitro (Kaminski and Stavnezer 2006). Importantly, that study further confirms an earlier report (Tarlinton et al. 1995) that, while B-1 cells can undergo isotype-switching to all downstream isotypes, their responses are clearly distinct from that of B-2 cells, resulting mainly in IgM and to a lesser degree in IgA production.

### 3.3.2 Active Contributions of B-1 Cells in Local Respiratory Tract Immune Defense

Given the early literature on the specific T-15-idotype encoded B-1a cell responses to S. *pneumoniae* infection outlined above (Kenny et al. 1983) and other studies that showed a strong induction of B-1 cell-derived auto-antibodies in a transgenic model following i.v. LPS injection (Murakami et al. 1994), our results were surprising to us at the time. More recent studies have also indicated the active involvement of B-1 cells, at least B-1b cells, in the systemic immune responses to *Borrelia hermsii* and *S. pneumoniae* infection (Alugupalli et al. 2004; Haas et al. 2005). Common to all published studies in which B-1 cell responses have been noted is the fact that the pathogen/LPS is administered systemically. In contrast, influenza virus infection is a localized infection of the respiratory tract. Therefore it appeared possible that B-1 cell responses to influenza virus infection might be induced in the respiratory tract but not systemically.

Ongoing studies are concerned with studying respiratory tract B-1 cell responses to influenza virus infection. Utilizing the same B-1/B-2 allotype-chimeric approach as in our previous studies, we now have experimental evidence that B-1 cells can indeed respond locally to influenza virus infection by accumulating in the draining lymph nodes and contributing to secretion of virus-binding and neutralizing IgM in the bronchoalveolar lavage (Y.S. Choi and N. Baumgarth, unpublished observations). Importantly, we have confirmed our original findings (Baumgarth et al. 1999) that systemic natural B-1 cell-derived antibody levels to influenza are unaffected, even in mice that have clear evidence of local B-1 cell responses. Thus, infection-induced local signals induce the accumulation and activation of B-1 cells in regional lymph nodes.

The mechanisms controlling B-1 cell migration following infections have not been studied in detail. CXCL13 was shown to be important for the homeostatic

migration/accumulation of B-1 cells into the peritoneal cavity and B-1 cell responses to peritoneal streptococcal antigen immunization (Ansel et al. 2002). Studies by Fagarasan and colleagues (Ha et al. 2006) indicate a role for TLR-dependent integrin and CD9-mediated migration from the peritoneal cavity following LPS stimulation. However, from that published study it can also be concluded that most migration from the peritoneal cavity is not dependent on CD9/MyD88 as most B-1 cells seemed to have left the peritoneal cavity following adoptive transfer into $RAG^{-/-}$ mice within a few hours after transfer (Ha et al. 2006). Similarly, most B-1 cells rapidly vacate the pleural cavity following adoptive transfer into wild type mice (Y.S. Choi and N. Baumgarth, unpublished observations). Thus, the continuous migration of B-1 cells into and out of the body cavities is further enhanced by innate immune signals such as TLR-mediated stimuli provided during an infection. Whether this innate stimuli alone is sufficient for the subsequent accumulation of these cells in spleen or regional lymph nodes and their differentiation to antibody-secreting cells remains to be investigated. It also remains to be studied whether B-1 cell subsets show differences in their ability to migrate from the cavities to sites of infection.

In summary, protective humoral immunity to pathogens is contributed by distinct B cell subsets with unique activation requirements and response patterns. The nature and tissue distribution pattern of the pathogen strongly affects the quality of the induced response. This is due at least in part to the induction of innate immune signals that provide additional regulatory stimuli to B cell responses and by triggering distinct B cell subset responses. A better understanding of the individual B cell subset response components that contribute during a natural infection might enable us to design better vaccines, which induce appropriate, strong, and long-lasting humoral immune responses.

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