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Division of labor during primary humoral immunity

Cristina L. Swanson,
Integrated Department of Immunology, University of Colorado, School of Medicine, Denver, CO, USA. National Jewish Health, 1400 Jackson Street, Denver, CO 80207, USA

Roberta Pelanda, and
Integrated Department of Immunology, University of Colorado, School of Medicine, Denver, CO, USA. National Jewish Health, 1400 Jackson Street, Denver, CO 80207, USA

Raul M. Torres
Integrated Department of Immunology, University of Colorado, School of Medicine, Denver, CO, USA. National Jewish Health, 1400 Jackson Street, Denver, CO 80207, USA

Raul M. Torres: torresr@njhealth.org

Abstract

B lymphocytes are often considered a homogenous population. However, B cells in both mouse and humans are comprised of distinct subpopulations that differ in development, phenotype, function, and microenvironmental niches. Much of our understanding about how these different B-cells populations mount antibody responses has been derived from experimental findings in mouse models and based on the use of model antigens. These reductionist studies performed over decades have been invaluable in defining the parameters of the B-cell antibody response to different types of antigens. However, these antigens also are now known to differ in a significant manner from *bona fide* physiological pathogens, and precisely how these different B-cell subsets divide labor in the primary humoral immune defense of pathogens is less well understood. While there are no absolutes in this area, there are recurring themes that divide the roles of B-cell subsets to different arms of the antibody response. This review provides an overview of rules that govern the B-cell labor roles, exceptions that break these rules, and models that have been used to define them.

Keywords

B-cell subpopulation; Follicular B cells; Marginal zone B cells; Primary antibody response

Introduction

Humoral immunity is a multilayered defense mechanism evolved for efficient control and elimination of pathogens. B-cell humoral immunity encompasses three distinct effector arms during infection: natural antibody function, extra-follicular ‘innate-like’ antibody responses, and germinal center antibody responses (Fig. 1). Natural antibodies are a component of the

Correspondence to: Raul M. Torres, torresr@njhealth.org.

innate immune system and are present prior to antigen exposure (Fig. 1) [1]. These pre-existing antibodies serve as a critical first line of defense against pathogens at the onset of infection [1–3]. Upon infection, a rapid acquired antibody response, also termed an extrafollicular, 'natural memory,' or 'innate-like' response, is induced within the first several days of antigen encounter [1, 4–6] (Fig. 1). This early acquired antibody response functions as a temporal bridge to control infection until more specialized antibody responses mature [5, 7]. Depending on antigen, approximately 1–2 weeks following infection, a delayed acquired antibody response is produced and again in an antigen-driven fashion [1, 8]. Albeit delayed, this classical adaptive humoral response is comprised by a selective assembly of highly efficient antibodies best suited for mediating clearance of the pathogen and protection from re-infection (Fig. 1).

Immunoregulatory parameters establish a division of labor

A division of labor has been established for anti-pathogen humoral immunity whereby distinct B-cell subpopulations dominate a particular facet of the antibody response. Regulatory parameters that dictate which B-cell subset comprises each arm of the humoral immune response include (1) the intrinsic responsiveness and homing patterns of unique B-cell subsets [5, 9]; (2) the dose, location, and timing of antigen encounter [10]; (3) the biochemical properties of the antigens themselves [9]; and (4) the induction of immunomodulatory innate signals [11].

Intrinsic properties of B-cell subsets

The peripheral pool of mature B cells in adult rodents is composed of three subpopulations including B-1, marginal zone (MZ), and follicular (FO) B cells. MZ and FO B cells are together classified as B-2 cells [12]. B-cell subsets are discriminated by their phenotypic expression of surface molecules, their dissimilar responsiveness to stimuli, and their location within different anatomical niches of lymphoid organs. These intrinsic properties unique to each B-cell subset are thought to influence the distinct contributions they make to humoral immune responses [5, 9].

B-1 cells—In rodents, B-1 cells are the first B-cell population to develop during ontogeny and reside within the peritoneal (PerC) and pleural cavities [13, 14]. Notably, however, while B-1 cells comprise the majority of PerC B cells, they constitute only ~1 % of the total B-cell compartment within the spleen [14]. Resident peritoneal B-1 cells are not constrained to the PerC as these cells have been reported to migrate from this site to the spleen, regional lymph nodes, and mucosal lamina propria during antibody responses to both infection (e.g., influenza virus) and systemically administered adjuvant (e.g., LPS) [13, 14]. Furthermore, B-1 B-cell migration to these sites has been shown to be dependent on CXCL13-directed migration [15, 16].

Murine B-1 cells are further classified into two major subpopulations: B-1a and B-1b cells. Both B-1 cell subsets rapidly differentiate into effector plasma cells upon stimulation and secrete antibodies predominantly of the IgM isotype, despite their potential to make IgG antibodies of all subclasses by polyclonal TLR stimulation in vitro [17]. Studies have framed a paradigm for distinct roles by B-1a and B-1b subsets in the antibody response,

whereby B-1a cells are the source of natural pre-immune IgM antibodies and B-1b cells produce acquired long-lived IgM and IgG3 antibody responses [3]. Despite these definitions, both B-1a and B-1b cells have also been appreciated to participate in numerous rapid antigen-dependent antibody responses such as to *Francisella tularensis* [18], *Borrelia hermsii* [19], *Streptococcus pneumoniae* [3, 4], and influenza virus [20].

The identity of a human orthologous population of mouse B-1 B cells remains uncertain although B cells with functional similarities to murine B-1 B cells have been identified [21–23].

MZ B cells—MZ B cells comprise approximately 5–10 % of the total B-cell population within the mouse spleen. They reside within the marginal zone of the spleen at the interface between the red and white pulp and adjacent to the marginal sinus where blood empties from arterioles [24–26]. As such, they are physically poised to be the predominant responding B cells to blood-borne pathogens. In rodents, MZ B cells are non-circulatory and confined to the splenic compartment under normal conditions [27, 28]. However, within the spleen, they are not sessile as they shuttle between the marginal zone and B-cell follicle to transport antigen to the white pulp in a naive state [29]. Furthermore, during immune responses, MZ B cells migrate to the junction of the T-cell zone and B-cell follicle (T-B border) within the splenic white pulp and even when responding to T cell–independent antigens [4, 30–32].

Naïve MZ B cells have also been characterized as ‘pre-activated’ whereby they are physically larger, constitutively express higher basal levels of surface activation molecules (CD69 and CD86), and have lower thresholds for activation than the major B-2 B-cell population, FO B cells [6, 24, 33]. Specifically, MZ B cells are more sensitive and responsive to activation by most stimuli including antigen-receptor signaling (anti-BCR), mitogens (e.g., LPS), and T cell-like help (e.g., anti-CD40+ IL-4) [34–38]. MZ B cells are also more efficient at priming naive CD4+ T cells than FO B cells, but a role for this interaction has remained to be elucidated during anti-pathogen responses [7, 32, 35].

In accord with a lower threshold for activation, MZ B cells respond and differentiate into plasma cells with accelerated kinetics to both bacterial and viral pathogens compared with other B-cell populations. An important outcome of a reduced threshold of activation and localization in the marginal zone is that MZ B cells mount rapid antibody responses after infection and directed to particular antigens.

MZ B cells in humans and mice share a number of surface markers [39], but, in contrast to rodent MZ B cells, human MZ B cells recirculate and are not confined to the spleen [26, 40]. Of note, human $IgM^{+}CD27^{+}IgD^{low}$ B cells are considered to represent splenic MZ B cells with a capacity to recirculate and, as such, are responsible for controlling infection to encapsulated bacteria and producing circulating anti-polysaccharide serum IgM [40–42], similar to murine MZ B cells [4].

FO B cells—FO B cells are the ‘conventional’ B-cell subset and constitute the vast majority of the total B-cell population in both humans and rodents. In humans, FO B cells are commonly referred to as the naïve B-cell population. These B cells circulate between the

blood and lymph and reside within B-cell follicles of the white pulp in secondary lymphoid organs. FO B cell–derived antibody responses are considered ‘classical’ adaptive humoral responses that require T cell–derived help and develop relatively slowly as they require maturation in germinal centers. Traditionally, these FO B-cell responses depend on signaling from (at least) both BCR engagement (signal 1) and T cell–derived help (signal 2; e.g., CD40-CD40L interaction and cytokines). The need for T cell help requires that before antigen-activated FO B cells can produce antibody, they first need to migrate to the T-B border and find an appropriate antigen-specific T cell with which to interact. Subsequently, FO B cells initiate germinal center reactions where they proliferate, somatically mutate their antigen receptor, and undergo class-switch recombination and memory B-cell formation [43, 44]. Thus, a consequence of these events is the temporal delay of FO B cells in generating antigen-driven acquired antibodies of high efficiency (Fig. 1). It should be noted, however, the capacity of FO B cells to also generate rapid antibody responses in regional lymph nodes has been newly appreciated for several pathogens including influenza virus [45] and *Salmonella typhimurium* [46].

BCR specificity—Both B-1 and MZ B cells are described as ‘innate’ lymphocytes in that they express a restricted repertoire of germline-encoded B-cell receptors (BCRs) for antigen recognition [47]. Importantly, these ‘innate’ B-cell subsets carry BCRs that express evolutionarily conserved VH gene segments that tend to be polyreactive, autoreactive, and of low affinity for antigens [47]. The BCR specificities of these B cells are enriched in ability to recognize conserved antigenic patterns common in numerous pathogens such as phosphorylcholine in the cell wall of Gram-positive bacteria (e.g., the T15 idiotype for B-1 cells and M167 idiotype for MZ B cells to *S. pneumoniae*) [47]. Upon infection, ‘innate’ B cells respond quickly to comprise the immediate acquired antibody response [4]. Thus, enriched specificity for a given microbial antigen within a B-cell population may also largely control which B cell subset dominates that particular response.

Antigenic properties

Early studies in the mouse initially classified antigens based on their ability to promote antibody production in the presence or absence of the thymus, and therefore T cells. Consequently, B-cell antibody-promoting antigens are now classified as either dependent on T cells (T-dependent; TD) or independent of T cells (T-independent; TI). TI antigens are further classified as either type 1 (TI-1) or type 2 (TI-2). TI-1 antigens are B-cell mitogens such as TLR ligands (e.g., LPS) that activate all B cells in a polyclonal manner through non-BCR signaling pathways. TI-2 antigens, on the other hand, present highly repetitive B-cell epitopes that are common to polymeric proteins or carbohydrates and that activate B cells through signaling that results from extensive BCR cross-linking.

We now understand that the nature of the B-cell antigen itself dictates T cell dependence and which B-cell subset is recruited into a response as well as the isotypes induced during the response. More specifically, the degree of organization, the number of epitopes displayed, and the biochemical constituents of the antigen all contribute to the type of B-cell response elicited [9, 48].

T cell–independent type 1 antigens—TI-1 antigens are mitogens that stimulate all B cells to produce antibody in a polyclonal manner and irrespective of antigen specificity [49]. Historically, TI-1 antigens were distinguished from TI-2 antigens by their ability to elicit T cell–independent antibody responses in CBA/N mice that displayed an X-linked immunodeficiency [50], later identified as a deficiency in the Btk tyrosine kinase. TI-1 antigens directly induce antibody production (mostly IgM) from all B cells without a requirement for either BCR signaling or secondary signals from accessory cells such as antigen-presenting cells (APCs) or T cells [49]. Physiological TI-1 antigens include TLR ligands, such as lipopolysaccharide (LPS) expressed by Gram-negative bacteria [51], or certain viral coat proteins [52–54].

T cell–independent type 2 antigens—TI-2 antigens are composed of repetitive epitopes displayed on a backbone that simultaneously engage multiple BCRs on the surface of antigen-specific B cells. In the mouse, these types of antigens elicit rapid B-cell responses dominated by IgM and IgG3 isotypes and in a T cell–independent fashion [55–59]. Although there is not a strict requirement for T cells for TI-2 antibody production, responding B cells have been observed to nevertheless migrate to the T-B border within the splenic white pulp during these responses [4, 30, 60, 61] and presumably to receive non-cognate T-cell help in the form of cytokines. Indeed, the presence of non-cognate T-cell help enhances production of other IgG isotypes, particularly of the IgG2a/ c subclass [55–57, 59]. Plasmablast growth and differentiation during TI-2 responses occurs with relatively accelerated kinetics and takes place mostly in extrafollicular locations (i.e., outside B-cell follicles) within either the bridging channels of the splenic red pulp or the medullary cords of lymph nodes [60, 62–64]. However, these antibody responses are comprised of low-affinity antibodies with limited isotype class-switching and the absence of long-term memory.

Biologically relevant TI-2 antigens include polysaccharides (PS) found on encapsulated bacteria such as *S. pneumoniae, Haemophilus influenzae,* and *Neisseria meningitides* [65]. Highly organized viral capsid proteins such as those found on the cytolytic vesicular stomatitis virus (VSV), poliovirus, and polyoma virus act as TI antigens as well [48, 54, 66–71].

In humans, the antibody response to the pneumococcal polysaccharide vaccines is dominated by IgG2 antibodies [72–74], which is consistent with the observed recurrent bacterial infections of individuals with IgG2 deficiency and their lack of an antibody response to immunization with *H. influenzae* type B polysaccharide capsule [75]. Similar to the mouse, the human antibody response to the DNP-Ficoll TI-2 antigen also results in rapid DNP-specific antibody responses that peak after 7 days and are comprised predominantly of IgM, IgG1, and IgG2 isotypes that remain at elevated levels for over a year [76]. Thus, rapid TI-2 humoral responses provide protective antibody during an important temporal ‘bridge’ between the early innate and late adaptive immune responses to pathogens [4] (Fig. 1).

It is now appreciated that the model antigens used to characterize the TI-2 antibody response differ in important ways from clinically relevant TI-2 antigens. For example, although hapten-coupled polysaccharides (e.g., Ficoll or dextran) have served to define much of our

understanding of TI-2 antibody responses, pathogen-derived TI-2 antigens will never be encountered in isolation. Rather, as bacterial polysaccharide capsules or viral capsids, pathogenic TI-2 antigens will virtually always be closely associated with TD antigens and, importantly, Toll-like receptor (TLR) ligands. Work from our lab has examined how previously characterized antibody responses differ in the presence of TLR ligands [11, 38].

T cell–dependent antigens—TD antigens are generally monomeric soluble proteins that display single or few epitopes to antigen-specific B cells [48]. TD antigens require both adjuvants and cognate T-cell help for induction of antibody production after immunization and again differ from *bona fide* TD antigens associated with pathogens that are able to induce antibody responses in the absence of adjuvant. In contrast to TI-2 extrafollicular responses, plasmablast expansion and maturation during TD responses typically occur in germinal centers located within B-cell follicles. During TD responses, B cells migrate to the T-B border where productive interactions between responding B cells and antigen-specific CD4+ T helper cells [77–80] lead to GC formation [8, 24]. Highly specific class-switched antibodies are generated in GC B cells by undergoing the diversification and selection processes known as somatic hypermutation (SHM), affinity maturation, and class-switch recombination [81]. Serum class-switched antibody levels during TD responses are detectable by 1 week after immunization, but high-affinity serum antibodies do not peak until approximately 2–3 weeks of the response [82]. TD antibody responses are dominated by IgG isotypes where the prevalent subclass of these isotypes is dictated by the specific adjuvant used in immunization or the cytokines elicited by pathogens. Overall, TD responses are considered ‘classical’ adaptive humoral responses characterized by slow kinetics and highly efficient, matured antibodies (Fig. 1).

Examples of TD antigens encountered during infection include bacterial toxin proteins such as tetanus and diphtheria toxoids, and unorganized viral envelope proteins [48, 54]. Hapten-linked protein antigens (e.g., NP-CGG, NP-OVA, Ars-KLH) are frequently used model antigens to study TD antibody responses in vivo.

Division of labor during TI-2 and TD antibody responses—A general division of labor is acknowledged between B-cell subsets and the response to TI-2 and TD antigens. B-1 and MZ B-cell populations have been considered to be primarily responsible for the antibody response to TI-2 antigens (Fig. 1) [4, 11, 30, 83]. Responding MZ B cells rapidly produce antibody at extrafollicular (non-GC) splenic sites early during TI responses that are of low affinity and predominantly IgM, but also include limited IgG subclasses [4, 84]. TI-2 antibody responses in the mouse resulting from immunization with hapten-polysaccharide model antigens have been characterized to be devoid of somatic mutation and long-term memory. However, we note that MZ B cells are capable of mounting initiating GC reactions and undergoing somatic hypermutation when directly examined, albeit with slower kinetics than FO B cells [31, 85]. Indeed, the antigen receptors expressed by rodent and human MZ B cells have been found to harbor somatic mutations [86], and the antibody response in humans to the capsular polysaccharide of *S. pneumoniae* is comprised of polysaccharide-specific antibodies with considerable mutations [87–89]. Thus, these data might suggest that in response to pathogen-derived TI-2 antigens, MZ B cells are able to undergo somatic

mutation and feasibly in germinal centers. As previously mentioned, *bona fide* pathogen-derived TI-2 antigens are intimately associated with both TD antigens and TLR ligands, which is not the case for hapten-polysaccharide model antigens that are delivered alone. Thus, although immunization with hapten-polysaccharides such as NP-Ficoll clearly does not elicit somatically mutated TI-2 antibody responses, this may possibly inaccurately reflect the response to bona fide TI-2 antigens as expressed by physiologically relevant pathogens. We suggest that re-visiting the antigen-specific antibody response to TI-2 antigens in the context of bacterial or viral immunogens may be warranted.

FO B cells dominate late acquired antibody responses to soluble protein TD antigens (Fig. 1). FO B cells provide high-affinity class-switched antibody generated from GCs and likewise seed the memory IgG B-cell compartment from this process. Conversely, the ability of FO B cells to directly participate in rapid extrafollicular TI-2 antibody responses has also been demonstrated, but is consistently a minor contribution compared with the MZ B-cell TI-2 response [90–92]. Although the division of labor established during humoral immunity is not unqualified, B-1 and MZ appear to be specialized for TI antibody responses whereas FO B cells are considered to be specialized to counter TD antigens [9].

Innate signals

It has been known for a long time that LPS, a TLR4 ligand, can polyclonally activate murine B cells to proliferate and secrete antibody [93, 94] and that MZ B cells are more sensitive to LPS activation than FO B cells [34–36]. More recent studies have documented variable expression of TLRs on the different mature B-cell subsets in both mouse and humans [17, 37, 95–98]. Thus, as pathogen infection not only presents TLR ligands but also leads to additional innate signals, it is important to define how these pathogen-derived innate signals differentially influence the different B-cell subsets during the in vivo antibody response. It is feasible that TLR signaling by B cells imparts useful information about the pathogen that then influences the type of antibody response.

During physiological anti-pathogen immunity, direct TLR or MyD88 signaling by B cells has been demonstrated during the neutralizing IgM response to VSV infection [99], the anti-PC IgG3 response to *S. pneumoniae* [100], and long-term IgG antibody production to polyoma virus [101]. Although still undefined, polyclonal IgM production in regional lymph nodes from B-1 cells early during influenza infection is likely mediated by innate signals [13, 20]. Innate signals also influence the isotype of the antibody produced during an immune response. Studies from our laboratory have recently demonstrated that innate signaling plays a key role in establishing a division of labor during humoral immunity [11]. Specifically, we showed that TLR-derived type I IFN signals FO B cells directly to recruit their participation in the TI-2 antibody response to NP-Ficoll and by the production of antigen-specific IgG2c. In the absence of type I IFNs, this response is largely dominated by IgM antibodies derived from MZ B cells [11].

Arms of the humoral immune response to foreign antigen

Natural antibodies

Natural IgM provides a critical systemic barrier against pathogen invasion prior to the induction of antigen-induced immune responses [2–4, 102–104]. Despite their low affinity for antigen, pre-existing IgM antibodies are able to directly neutralize and control early pathogen replication in part due to their broad cross-reactivity and high avidity [2, 102–104]. B-1a cells are responsible for generating the vast majority of pre-existing natural serum IgM antibodies [1] (Fig. 1), and B-1a cell-derived natural IgM has been shown to be essential for early control and subsequent clearance of both influenza virus [1, 2] and *S. pneumoniae* infections [3].

Extrafollicular bridge antibody responses

Generation of protective antibody titers during host responses is a vital early defense mechanism to fighting exponentially replicating pathogens [66]. It is of clear advantage to the host to rapidly establish these titers at the onset to control infections, and accordingly, these responses have been observed in numerous pathogen models including influenza virus. For instance, during humoral immunity to influenza virus, a localized IgG2c extrafollicular B-cell antibody response is detectable within several days in mediastinal lymph nodes following intranasal infection [105]. Similarly, VSV has been demonstrated to have a highly organized antigenic capsid capable of eliciting T cell–independent antibody responses [67], while simultaneously inducing an early isotype-switched antibody response [48]. These early class-switched responses are not exclusive to antiviral responses as *S. typhimurium* also promotes an extrafollicular IgG2c class-switched plasma cell response detectable 4 days after infection [46].

Rapid extrafollicular antibodies generated during immunity to both model antigens and pathogens are largely derived from B-1 and MZ B cells. MZ B cells, together with B-1 cells, participate in the early stages of the T cell–independent IgM antibody response to the phosphorylcholine (PC) antigen that is situated within the bacterial cell wall of *S. pneumoniae* [4]. MZ B cells also generate rapid IgG antibodies that peak at day 6 to the capsular polysaccharide PPS14 within the bacterial capsule of *S. pneumoniae*, although T cells are required for this response [9, 84].

Rapid MZ B-cell responses are also observed in antiviral responses where these cells expand and differentiate to class-switched effector cells in the early phase of the response [106, 107]. For influenza virus, the acquired virus-induced component of the humoral response consists of both systemic B-2 cell-derived IgM and IgG2a [1, 2], in addition to a rapid and localized lymph node IgG response by FO B cells [45].

Although less appreciated, B-1a cells likewise participate in rapid antigen-driven humoral responses (Fig. 1) [18]. B-1a cells mount an anti-LPS IgM response to *F. tularensis* within 3 days of infection and independent of T-cell help [18]. Conversely, B-1b cells strictly participate in antigen-dependent antibody responses and contribute to both rapid and long-term protective humoral immunity (Fig. 1). Within the first several days of infection, B-1b

cells mount accelerated IgM responses to *B. hermsii* [19], and IgM and IgG3 responses to *S. pneumoniae* polysaccharide antigen PPS-3 [3].

The response to the TI-2 model antigen, NP-Ficoll, has been characterized by the domination of IgM and IgG3 isotypes derived from MZ B cells [108–111]. This generalization has been drawn through two lines of investigation using both splenectomy models [11, 112] and MZ B cell–deficient knockout mouse models [108–111, 113–115]. While the capacity of lymph node B cells (i.e., FO B cells) to respond to NP-Ficoll has been shown [90–92, 116], their role in TI-2 antibody responses is typically limited with respect to other B-cell subsets [4, 30] unless in the presence of the type I interferon innate signal [11].

Germinal center-derived antibody responses

The FO B-cell antibody response to a TD antigen proceeds through a germinal center reaction and represents one of the most highly evolved manifestations of adaptive humoral immunity. In addition to producing long-term B-cell memory, these responses result in highly specific, somatically mutated antibodies bearing class-switched immunoglobulin best suited for countering the offending pathogen. However, the formation and maintenance of germinal centers requires the productive physical interaction between antigen-specific B and T lymphocytes before and during the germinal center reaction, the clonal expansion of B cells receiving T cell help, somatic mutation and selection of class-switched germinal center B cells, and ultimately the differentiation of these responding B cells into either an antibody secreting cell or memory B cell [43, 44]. These cellular interactions depend on lymphocyte migration to appropriate microenvironments and the subsequent execution of complex cellular processes (e.g., somatic mutation, class-switch recombination). Thus, this arm of humoral immunity, while highly effective, takes time to establish and is considerably delayed relative to the rapid extrafollicular bridge antibody responses. Thus, the evolution of germinal center-derived antibody responses has only been possible due to the division of labor in humoral immunity in which MZ and B1 B cells control early phases of pathogen infection.

Germinal centers are formed in B-cell follicles in secondary lymphoid organs, and many of the chemoattractants that orchestrate cell migration into and within the germinal center have been defined [43, 44]. The molecular basis for the fate of a responding B cell as either a memory B cell or long-lived plasma cell is ill-defined but intensely investigated. As discussed above, MZ (and B1) B cells are not considered to initiate or participate in germinal-center reactions although when directly tested they are able to both form germinal centers and somatically mutate their antigen receptors [31, 85].

Vaccines

The major goal of the currently used vaccines in medicine to date is to generate highly specific, class-switched antibodies with long-term memory. Accordingly, these vaccines are designed to specifically target FO B cell, germinal center–derived antibody responses. This approach has been successful as vaccines now prevent or significantly reduce a large number of important diseases. However, it has also become evident that this strategy is not universally applicable to preventing disease as a number of pathogens are refractory to

similar vaccine strategies and remain global health burdens. We argue that with increasing understanding of both pathogen biology and the division of labor between different B-cell subsets in humoral immunity, future vaccine strategies should be able to selectively call upon these different subsets in ways that best counter the offending pathogen. Thus, future studies of humoral immunity in response to adjuvants and pathogen-associated molecular patterns should also focus on understanding how innate signals, in the context of different types of B-cell antigens, polarize antibody responses differently from Alum and in order to provide more effective immunity. As an example, type I IFNs have been shown to accelerate both the class-switched TI-2 response [11] and to enhance GC formation and affinity maturation during TD responses [117, 118]. This knowledge, along with our understanding of the B-cell division of labor, should be harnessed to collectively tailor vaccines to preferentially call the B-cell subsets best suited to generate protective humoral immunity during human vaccination, and of the appropriate isotype.

Concluding remarks and future questions

B lymphocytes belong to distinct subsets that display differential antibody repertoires, functional properties, and residential microenvironmental niches. Rodent studies have been invaluable in defining how the different B-cell populations contribute to the antibody response to different types of model antigens. However, it is understood that these model antigens differ in important aspects from physiological pathogens, and it will be important in the future to determine how, for example, the antibody response to TI-2 antigens differs when encountered in isolation versus presented by pathogens. While much has been learned about how each B-cell subset contributes to the different arms of humoral immunity, it will also be important to further define the parameters of how each subset participates in the primary antibody response. Current lines of investigation in mouse models suffer because the B-cell phenotype that identifies subset membership is usually altered or lost relatively soon after activation and during the course of the response (unpublished observations). Thus, even with the ability to identify antigen-specific B cells during the primary antibody response either flow cytometrically or histologically, it becomes challenging to determine the origin of the B-cell subset from which these antigen-specific B cells originate. In addition, while mouse strains with engineered mutations leading to deficiencies in specific B-cell subsets have been used to characterize the role of the missing B-cell population, the remaining B cells nevertheless harbor these mutations and likely skew their function. Thus, an ideal animal model would (1) facilitate identifying which components of the antibody response are derived from distinct B-cell subsets, (2) harbor B cells with a wild-type BCR repertoire capable of responding to all antigens, and (3) have minimal alteration to the function of other B-cell subsets or other accessory cells. With such an animal model, it should be feasible to determine the precise contribution of different B-cell populations to different foreign antigens. Future vaccine strategies should then selectively exploit the biology of B lymphocytes and their subset to elicit the antibody response best at countering the pathogen of interest.

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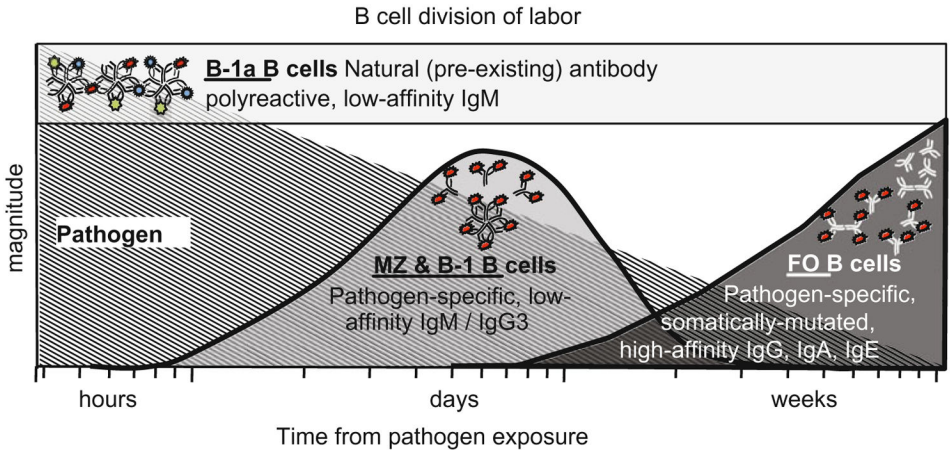

Fig. 1.

Humoral immunity to pathogen exposure is accomplished by a division of labor between B-cell populations. Humoral immune control of pathogens is composed of three arms of antibody responses that are depicted as a function of the kinetics and magnitude of the response. Natural IgM composes the innate arm and is derived from B-1a cells prior to antigen exposure. Bridge immunity encompasses rapid TI-2 antibody responses predominantly derived from MZ B and B-1 cells. Slower adaptive humoral responses are largely contributed by FO B cells and characterized by highly matured antibodies generated in germinal centers through somatic hypermutation, affinity maturation, and class-switch recombination