Innate-Like B Cells and Their Rules of Engagement

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Abstract

Antibodies are an integral part of the immune system. They are produced in response to an infection or insult but are also present prior to any encounter with antigen as so-called natural antibodies. This review focuses on the tissues and cellular origins of natural antibodies. It summarizes recent data showing that B-1 cells, an innate-like B cell population distinct in development, repertoire, and tissue location from the majority conventional or B-2 cells, are the main contributors of natural antibodies in mice in steady state. Furthermore, they show that natural IgM production appears largely confined to B-1 cell populations in the spleen and bone marrow. In contrast, B-1 cells in the body cavities, sites of predominance of this population, harbor B-1 cells that do not constitutively produce antibodies. Instead, these cells act as rapid immune responders that relocate to secondary lymphoid tissues and differentiate to cytokine and antibody-secreting cells shortly after an infection. Thus, the process of B-1 cell response participation is distinct from that of B-2 cell activation as the accumulation of effector B-1 cells does not rely on extensive clonal expansion, but instead on their rapid migration and redistribution, a process that appears under the control of infection-induced innate signals.

Keywords

Antibody secretion • B-1 cells • Body cavities • Natural IgM • Innate-like responses

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7.1 Introduction

The immune system is an exquisite and rapid response system. Specific antibody production is induced rapidly, sometimes in less than 2–3 days following encounter with a pathogen or a noxious agent. These antibodies are generated after

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conventional bone marrow-derived B cells have bound to pathogen-derived antigens via surface antigen receptor (B cell receptor, BCR), which induces their clonal expansion and differentiation to antibody-secreting plasma blasts and plasma cells. These activation and differentiation pathways are increasingly well mapped (reviewed in [1]). They often involve interaction of the responding B cells with antigen-presenting cells and CD4 T helper cells and occur in specialized secondary lymphoid tissues optimized to enhance potential interaction with these cells [2]. Induction of these adaptive immune responses also leads to long-term changes of the humoral immune system, causing the development of memory B cells and long-lived plasma cells. The latter produce specific antibodies continuously for years, if not decades, after that initial insult.

While conventional B cells do not generate antibodies unless stimulated, serum antibodies and antibody-forming cells are readily found prior to encounter with pathogens, and even in gnotobiotic mice held in the complete absence of antigens [3–5]. In fact, much of the circulating IgM serum antibodies are "natural antibodies." These antibodies do not require foreign antigenic stimulation and thus the signaling and differentiation pathways that induce natural antibody production must be distinct from those inducing antigen-specific B cell responses. Little is known about the regulation of natural antibodies and natural antibody-secreting cells. Identification of these processes is of significance, however, as natural antibodies provide crucial immune protection against both viral and bacterial pathogens [6–9].

Here I will provide a summary of recent data from our group and others that show that B-1 cells, a cell population distinct in development, repertoire, and tissue location, are the main contributors of natural antibodies in mice in steady state and that natural IgM production appears confined largely to B-1 cell populations in the spleen and bone marrow. In response to influenza virus infection, antibody-producing B-1 cells are activated and effector cells redistribute without extensive clonal expansion to secondary lymphoid tissues, a process that seems controlled by innate-like signals.

7.2 B Cells and Their Contribution to the Antibody Pools

Based on developmental origins, B cells can be divided into two lineages: B-1 and B-2. B-1 cells appear first in ontogeny, hence their name B-1, and are derived from distinct precursors found in the splanchnopleura of developing embryos, the fetal liver, and the bone marrow. The latter precursors do not contribute significantly to B-1 cell pools during adulthood, unless stimulated as occurs during severe lymphopenia, such as after sublethal or lethal whole-body irradiation (reviewed in [10]). This reactivation of B-1 cell development after irradiation has contributed to the earlier debates about the origins of B-1 cells. However, the recent studies by Montecino-Rodriguez and colleagues [11, 12], showing distinct origins and developmental periods of B-1 and B-2 cell development, are consistent with many of the early data on B-1 cells that suggested a restricted developmental period for B-1 cells in mice that spans the early fetal life until 3–6 weeks after birth. Furthermore, the isolation of precursors with B-1 cell-restricted potential clearly supports the different lineage origins for B-1 and B-2 cells.

Bone marrow B-2 cell precursors give rise to both follicular B cells and marginal zone B cell populations. Follicular B cells are the main populations responding to antigen exposure with formation of germinal centers and thus long-lived responses. They are the majority B cell population in the host and are predominant in all major lymphoid tissues. Marginal zone B cells in the mouse are restricted to the splenic marginal zone, while their counterparts in humans appear to be found also circulating in the blood. MZ B cells respond rapidly to blood-borne infections with formation of extrafollicular foci, which harbor short-lived plasma cells. While MZ B cells can also participate in germinal center responses, their contributions to these centers appear to be small and to occur only late after immunization [13].

B-1 cells (previously known as Ly-1 B cells or $CD5^+$ B cells) are divided into two subsets, B-1a and B-1b, based on their expression of CD5. $CD5^+$ B-1a cells and $CD5^-$ B-1b cells seem to

share developmental precursors. Apart from the differential expression of CD5 they are phenotypically similar, and clear functional differences are also not apparent. They do seem to differ, however, in the type of antigen or infectious agent they respond to. For example, in mouse models of *Streptococcus pneumonia,* B-1a and B-1b cells seem to recognize and respond to distinct antigens on the bacteria [14]. During infections with the relapsing fever agent, *Borrelia hermsii*, B-1b but not B-1a cells contribute to IgM-mediated humoral immunity during recall responses [15], while in influenza virus infection only B-1a cells respond to the infection with redistribution to local draining lymph nodes and IgM production [16].

B-1 cells are rare in secondary lymph nodes (about 0.2–0.3%), and represent only about 1% of splenic B cells. They are, however, the major B cell population in the peritoneal and pleural cavities (reviewed in [17]). Depending on the strain, age, and sex of the mouse, B-1 cells comprise 30–60% of total lymphocytes at these sites. Due to their unique tissue-distribution and low frequencies in other tissues, most information about B-1 cells is based on studies with peritoneal cavity (PerC) B-1 cells. However, as we will outline below, differences exist between body cavity and spleen B-1 cells, which remain to be fully evaluated.

The distribution of B-1 cells is unusual and distinct from that of B-2 cells and suggests significant functional differences between B-1 and B-2 cells. Indeed, studies with chimeric mice, created by treating newborn mice with host-allotype-specific IgM to deplete host B-1 cells and reconstituting them with congenic but allotype-mismatched B-1 cells, demonstrated that B-1 cells are the major source of natural IgM in the serum of noninfected mice [8, 18] and in the mucosal tissues of the intestinal [19] and the respiratory tract [16]. Thus, B-1 cells appear to be uniquely positioned to produce antibodies in the absence of foreign antigenic signals. Understanding the biology and physiology of their responses and the regulation of their antibody production is thus likely to uncover also the regulation of natural antibody production.

7.3 Role and Function of Natural Antibodies

Natural antibodies are important in host defense. Numerous studies showed that following infections with both viral and bacterial pathogens, preexisting IgM antibodies directly neutralize and inhibit early pathogen replication, in part via complement binding, and thereby increase survival from infection [6, 9, 20–23]. With mice lacking secreted IgM, it was shown that the presence of natural IgM enhances the ensuing pathogen-specific IgG responses [21, 24], possibly via the formation of antibody–antigen complexes for their deposition on follicular dendritic cells [21, 25], although other mechanisms are likely [25]. Analogous "natural" poly-specific IgA antibodies exist at mucosal surfaces, where they might act as a first layer of immune defense [19, 26]. Thus, natural antibodies constitute an important component of preexisting protective immunity. These antibodies are often polyreactive, meaning they will bind to multiple antigens. This interaction is usually a low-affinity interaction ($Kd = 10^{-3}$–10^{-7} mol l^{-1}); however, given the pentameric structure of the IgM, overall affinities may nonetheless be considerable [27], supported by data on their protective effects on host survival following infections.

Another function of natural antibodies is their involvement in "housekeeping" functions: the maintenance of tissue integrity and homeostasis. They facilitate removal of apoptotic cells via their binding to surface antigens such as phosphatidylcholine (PtC), annexin IV [28], phosphorylcholine [29], and malondialdehyde, the latter a reactive aldehyde degradation product of polyunsaturated lipids [29–32] and xenoantigens [33]. This seems to facilitate increased phagocytosis by immature dendritic cells [31], while also limiting tissue inflammation [31]. The genetic ablation of secreted IgM results in increased autoimmunity, with accelerated, pathogenic IgG responses and resulting disease progression [34], further suggesting that IgM participates in the removal of self-antigens to lower the likelihood of autoimmune responses. Inappropriate and/or

enhanced local secretion of natural IgM secretion and ensuing IgM-self-antigen complex formation can result in local activation of the complement cascade and tissue damage, as seen during ischemia-reperfusion injury [28, 35]. Natural antibody binding to self-antigens seems to be involved also in atherosclerosis development, where these antibodies contribute to plaque formation via their binding to oxidation-specific epitopes on low-density lipoproteins and cardiolipins [29, 32]. Thus, natural antibody secretion and activation must be carefully controlled to ensure their beneficial effects, while avoiding the potential dangers of their inappropriate activation.

7.4 Tissue Origins of Natural Antibodies

Early studies by Benner and colleagues followed the development of spontaneous antibody production in gnotobiotic and SPF-housed mice and demonstrated the largely antigen and T cell-independent development of spontaneous IgM-secreting cells in two tissues: the spleen and the bone marrow [36, 37]. However, their phenotype and lineage origins were not defined at that time, and the body cavities were not investigated as sources for natural antibody-producing cells. In support of the early studies by Benner, natural IgM-secreting B cells were shown by others to be present in the steady-state spleen [38, 39]. Erythrocyte-autoantibody transgenic mice lack splenic B-1 cells [40, 41] and B-1 cells were the major if not the only source of natural IgM in the spleen of Ig-allotype-chimeric mice [18].

Since B-1 cells are the predominant B cell subsets in the peritoneal cavity (PerC), natural IgM was thought to originate from there [7, 42]. Recently Rothstein and colleagues further suggested significant spontaneous IgM secretion by PerC B-1 cells [43]. A number of studies indicate, however, that PerC B-1 cells do not spontaneously produce natural IgM, either in vivo or ex vivo [38, 39, 44]. Instead, the data suggested that activation signals such as cytokines (IL-5 and IL-10) or mitogenic activation with LPS activated IgM secretion by PerC B-1 cells [40, 41]. Indeed, injection of bacteria or LPS into the PerC did not induce antibody production in the PerC. Instead it caused the migration [45] and differentiation [46] of PerC B-1 cells to IgM-secreting cells in the spleen via TLR4-mediated activation [45]. The spleen and the bone marrow are also sites of long-lived antibody production by B-2 cell-derived plasma cells following vaccination or infection [47, 48].

Because of these discrepancies in the literature and the importance of univocally identifying the B cells that produce natural antibodies, we recently revisited the question of the tissues and cellular origins of spontaneous IgM secretion [49]. For that we screened various tissues, including PerC, spleen, bone marrow, and lymph nodes for the presence of spontaneously IgM-secreting cells via allotype-specific ELISPOT. The results demonstrated that spleen and bone marrow, but not PerC (or pleural cavity) cells, contained large numbers of IgM-secreting cells, prior to immunization or infection. Since the bone marrow had never been shown to harbor B-1 cells, we then sought to identify the phenotype of these secreting cells. We demonstrated that spontaneous IgM-secreting cells were enriched among $CD19^{hi}$ $CD43^{+}$ IgM + cells, a phenotype consistent with that of B-1 cells. Further analysis showed that bone marrow B-1 cells resembled their counterparts in the spleen, but not the PerC by phenotype, as they lacked CD11b expression, a hallmark of body cavity B-1 cells, and they were IgM^{hi} IgD^{lo} $CD19^{hi}$ $CD43^{+}$. Finally, studies using allotype-chimeric mice confirmed that most of the spontaneous IgM-secreting cells in both spleen and bone marrow are B-1 cell derived. Remarkably, when B and T cell-deficient $RAG^{-/-}$ mice were reconstituted with bone marrow depleted of IgM + cells, thus depleting all B-1 cells, these mice had no detectable levels of serum IgM 6 weeks after reconstitution, compared to mice reconstituted with complete bone marrow, despite significant reconstitution of their B cell populations [49]. This data thus demonstrate that the bone marrow niche harbors IgM-secreting B-1 cells, which together with splenic B-1 cells are major contributors of serum IgM. The transfer of adult IgM^{-} bone marrow precursors, however, does not easily replenish these cells.

Collectively the data suggest that while B-1 cells are most prevalent in the body cavities, PerC cells do not appear to spontaneously secrete IgM. In contrast, B-1 cells in bone marrow and spleen, two major sites also of B-2 cell-derived plasma cells, instead support the production of natural IgM by B-1 cells. While body cavity B-1 cells do not generate steady-state natural IgM, they can nonetheless be rapidly activated to migrate to the spleen, and possibly the bone marrow, although the latter has not been experimentally demonstrated, to differentiate to IgM-secreting cells. Whether the repertoire of the splenic and bone marrow natural IgM is similar to that of IgM secretion by body cavity B-1 cells following their activation is currently unclear. Earlier studies on B-1a cells' ability to bind to PtC, visualized with PtC-liposomes by flow cytometry, showed higher frequencies of PtC-binders among the PerC than spleen B-1 cells. Clearly, more work is required to investigate the relationship between truly "natural" IgM-secreting B-1 cells and those that can be induced to differentiate and secrete IgM in response to an infection or other outside stimulus.

7.5 Regulation of Natural Antibody Production

The molecular mechanisms governing B-1 cell differentiation to antibody-secreting cells are somewhat controversial. Studies with B-2 cells have revealed the main regulators of the B cell differentiation pathway (reviewed in [1]). In B-2 cells, BLIMP-1 (B lymphocyte-induced maturation protein) induced during differentiation to Ig(M)-secreting plasma cells [50–52] is thought to suppress the transcriptional repressor PAX-5 (paired box protein) [53–55]. Removal of PAX-5 induces activation of XBP-1 (X-box-binding protein). XBP-1 and spliced XBP-1 (sXBP-1) regulate the unfolded protein response in the endoplasmic reticulum of antibody-secreting plasma cells [56, 57]. Ectopic expression of either BLIMP-1 or XBP-1 in B cell lines and primary B cells drives plasma cell differentiation [53, 57, 58].

The role of BLIMP-1 in the differentiation of B-1 cells to natural IgM-secreting cells is less clear. Calame and colleagues reported that BLIMP-1 is essential for B-1 cell-derived natural IgM production and protection from influenza infection, but is dispensable for the development and self-replenishment of B-1 cells in BLIMP-1 knockout mice [59]. In support of these findings, Tarlinton and colleagues reported that B-1 cells in the PerC express low levels of BLIMP-1 and do not spontaneously produce IgM, but could be induced to express BLIMP-1 and secrete IgM after LPS-stimulation [44]. In contrast, Tumang et al. concluded that B-1 cells do not depend on BLIMP-1 expression to differentiate into natural IgM-secreting cells in vitro as they found IgM production, but not BLIMP-1 expression by PerC B-1 cells [43].

The studies by Tumang et al. [43] were done with PerC B-1 cells, which they suggested produce IgM, but which we and others do not find to spontaneously produce significant amounts of IgM [38, 39, 44, 49]. While the reasons for these discrepant findings with regard to PerC B-1 IgM secretion are unknown, it is possible that the process of isolating B-1 cells from the PerC may cause their rapid activation and differentiation. This could explain spontaneous IgM secretion by PerC B-1 cells, possibly influenced by the method of isolation. In support, we observed that while total PerC cell cultures result in little IgM production when plated straight from harvest onto ELISPOT plates, aliquots of the same PerC cells, but stained and B-1 cells then sorted by FACS, will show enhanced IgM secretion, even in the absence of significant BLIMP-1 expression, as measured with cells from BLIMP-1 reporter mice (unpublished observation). Also, this "spontaneous" IgM secretion by PerC B-1 cells does not occur within the first day of culture, but increases with time of culture, indicating nonspecific activation in the cultures (unpublished observations). In addition, an earlier study had shown that the presence of prostaglandin (PG) E_2-producing macrophages can inhibit differentiation and IgM secretion of B-1 cells in the PerC [60] and that LPS stimulation of macrophages increases PGE_2

production. This would further suggest that isolation of B-1 cells may not only lead to their stimulation, the removal of PGE_2-producing cells during the isolation procedure may further enhance their spontaneous differentiation and IgM production.

Collectively, these studies indicate to us that B-1 cell in the body cavities are not contributing directly to the steady-state pool of natural antibodies, but that they can be stimulated rapidly to produce IgM. Indeed, as discussed above, multiple studies have shown the rapid relocation of PerC B-1 cells to the spleen following exposure to cytokines or mitogenic signals, consistent with the notion that PerC B-1 cells are ready to rapidly respond to an insult. Furthermore, it is possible that they but may not always fully differentiate to plasma cells to do so. Thus, most studies support the notion that the body cavity B-1 cells act like "armed soldiers" ready to go into combat zones (spleen or lymph nodes, possibly also mucosal sites) upon an infection-induced signal. While TLR4 has been shown to be one such signal, it is likely that other innate signals can have similar effects, based on the type of infection the host is encountering. This infection-induced production of body cavity-derived B-1 cell IgM must not be confused with the steady-state production of natural antibodies, mainly in bone marrow and spleen, which do not require outside signals for their production. Having identified true "natural" IgM-producing cells in the bone marrow of mice, their molecular characterization can now be used to define the differentiation stage and molecular mechanisms underlying natural antibody production.

7.6 B-1 Cell Migration

The scenario outlined above indicates that one important outcome of B-1 cell activation is their altered migratory behavior. However, the mechanisms of their migration to and from the body cavities and between body cavities and secondary lymphoid tissues are incompletely understood. B-1 cells are readily found in the blood of mice, where they represent about 0.5% of B cells; thus redistribution of B-1 cells via the bloodstream is a possible mechanism of their distribution throughout the body and into the body cavities. In support, Cyster and colleagues showed that mice deficient in the chemokine CXCL13 lack B-1 and B-2 cells in the body cavities while the numbers of B-1 cells in the blood of these mice were significantly elevated compared to wild-type mice [61]. The spleen populations appeared unaffected. Further support for considerable recirculation of B-1 cells into and out of the body cavities was provided in the same study with parabiosis experiments. Two months after fusion of the blood circulation between two mice, there was significant mixing of B-1a and B-1b cells in the PerC, albeit not as complete as that of B-2 cells. Thus, B-1 cells can circulate to and from body cavities, possibly via entry through the blood and omentum [61] in adult mice. It is likely that the increased migration of PerC B-1 cells, observed following TLR4-mediated activation, represents an enhancement of this otherwise rather slow recirculation process. Importantly, in the latter case, B-1 cells seem to lodge themselves into the spleen where they begin to secrete antibodies. Thus, activating signals seem to (a) enhance the migration of B-1 cells from the body cavities and (b) facilitate the enhanced entry of activated B-1 cells into secondary lymphoid tissues and (c) initiate their differentiation (Fig. 7.1).

7.7 Innate-Like B Cell Responses to Influenza Virus Infection

To study the responses and contribution of B-1 cells during an immune response, we began a number of years ago to focus on B-1 cell responses during influenza virus infection. Influenza virus infection in humans and other mammals is typically a respiratory tract infection, transmitted via droplets into the upper respiratory tract. Epithelial cells in the nasal cavity and/or upper pharynx are likely the first targets of seasonal influenza virus strains, although more pathogenic strains might directly infect lower parts of the lung. The local draining lymph nodes act as major sites of B cell response induction following influenza infection

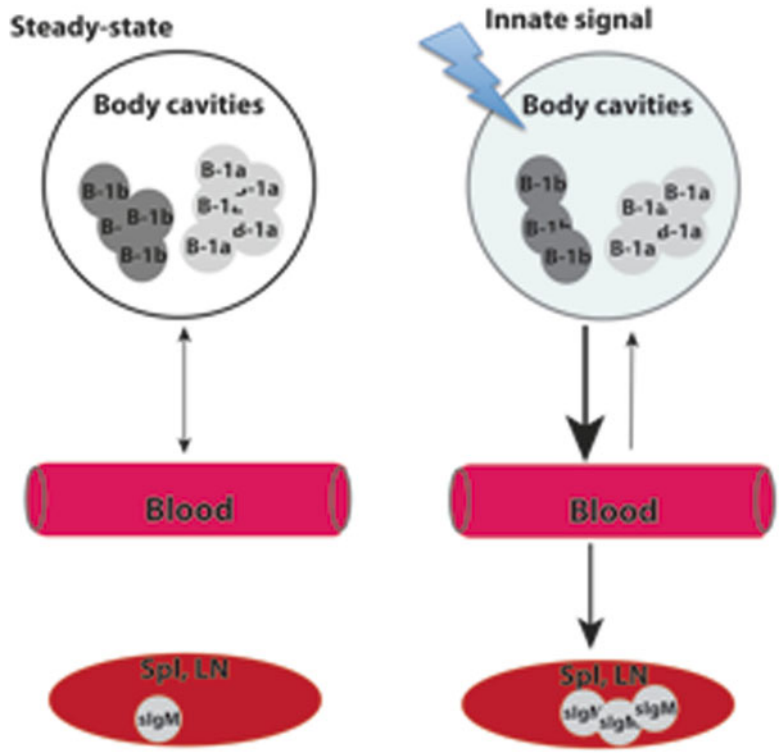

Fig. 7.1 Alteration in B-1 cell migration following an infection or insult. In steady state (*left*) there is a slow migration of B-1 cells into and out of the pleural and peritoneal cavity, likely via the omentum and similar structures in the pleural cavity into the blood. From there B-1 cell may recirculate back into the body cavities. IgM-secreting B-1 cells are also found in the spleen (and bone marrow, not depicted), providing steady-state natural IgM. In response to an infection (*right*), B-1 cell rapidly leave the body cavities, in the short-term lowering the numbers of B-1 cells at those sites. The cells then redistribute to spleen and other secondary lymphoid tissues, where they differentiate to IgM-producing cells

[62, 63]. In both mice [16, 63, 64] and ferrets [65], the gold standard influenza virus infection animal model, antibody-secreting cells are identified as early as 3 days after initial infection in the cervical and mediastinal lymph nodes as antibody titers rise in nasal washes and lung lavages. Antibody-secreting cells are identified in the cervical and mediastinal lymph nodes of mice and ferrets, as early as 3 days after initial infection [62, 63]. Since mice are usually infected directly into the lung tissues, lower respiratory tract draining mediastinal lymph nodes are the main secondary lymphoid tissues involved in the response.

Initial studies by us and others showed that B-1 cells contribute to protection from influenza virus infection even prior to any encounter with the virus by generating protective IgM antibodies that are generated constitutively in the absence of influenza exposure [8, 20, 66]. More recently, studies on their responses to influenza infection highlighted the ability of B-1 cells, specifically B-1a cells, to respond to infection in an innate-like manner. $CD5^+$ B-1a cells, but not $CD5^-$ B-1b cells, increased in frequency locally in the draining lymph nodes during acute influenza virus infection (days 5–10) and they secreted increased amounts virus-binding IgM into the airways [16], consistent with the above-outlined migration after B-1 cell activation. Importantly, influenza virus-binding IgM represented only a small fraction (roughly 10%) of the overall increases in IgM production by B-1a cells in lymph nodes and airways, and the relative amounts of influenza-binding

natural antibodies did not increase over time compared to non-influenza-binding IgM. This apparent lack of clonal expansion was further supported by BrdU-labeling studies, which indicated a complete lack of B-1 cell clonal expansion over the course of the infection [16].

Thus, B-1 cells respond to influenza infection with redistribution to and differentiation at the site of infection, while maintaining steady-state levels natural serum antibody levels [8, 16, 20]. It is tempting to speculate that the redistribution of B-1 cells, which our data show occurs at least in part from the pleural cavity, is a consequence of systemically elaborated innate cytokines. Consistent with this, influenza infection-induced type I IFN can profoundly affect leukocytes at distant sites [67], and our data thus far with type I IFNR$^{-/-}$ mice support a role for IFN in regulating B-1 cell migration via the activation of surface integrins (Waffarn, EE. Hastey, CJ, Dixit, N., Simon SI, and Baumgarth, N, in preparation). While we expected that the B-1 cell migration would result in the accumulation of lung IgM-secreting B-1 cells, we did not find evidence for that. Instead, the major accumulation of IgM-secreting B-1 cells occurred in the draining mediastinal lymph nodes [16], raising the question what additional functions, apart from IgM secretion, B-1 cells might play during the initiation of the adaptive immune response in the draining lymph nodes. There is evidence that B-1 cells and secreted IgM might contribute to immune protection against influenza other than by virus neutralization [16, 20, 22]. For example, B-1 cell-derived IgM is required for maximal induction of (B-2 cell-derived) influenza virus-specific IgG [20]. B-1 cells are also known as strong producers of IL-10 [68] and GM-CSF [69] thus could be involved in the regulation of local immune responses, similar to a recently identified regulatory role proposed for a B cell subset that shares some phenotypic characteristics with B-1 cells [70]. Increasing our understanding on the mechanisms that regulates B-1 cell migration and their function might help the development of therapies or prophylaxes to garner the help of these fascinating lymphocytes in immune protection.

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