Immunol Res (2015) 63:153–166
DOI 10.1007/s12026-015-8708-3

AUTOIMMUNITY/IMMUNOREGULATION/INFLAMMATION

The role of B-1 cells in inflammation

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Published online: 1 October 2015

Abstract B-1 lymphocytes exhibit unique phenotypic, ontogenic, and functional characteristics that differ from the conventional B-2 cells. B-1 cells spontaneously secrete germline-like, repertoire-skewed polyreactive natural antibody, which acts as a first line of defense by neutralizing a wide range of pathogens before launching of the adaptive immune response. Immunomodulatory molecules such as interleukin-10, adenosine, granulocyte–macrophage colony-stimulating factor, interleukin-3, and interleukin-35 are also produced by B-1 cells in the presence or absence of stimulation, which regulate acute and chronic inflammatory diseases. Considerable progress has been made during the past three decades since the discovery of B-1 cells, which has improved not only our understanding of their phenotypic and ontogenic uniqueness but also their role in various inflammatory diseases including influenza, pneumonia, sepsis, atherosclerosis, inflammatory bowel disease, autoimmunity, obesity and diabetes mellitus. Recent identification of human B-1 cells widens the scope of this field, leading to novel innovations that can be implemented from bench to bedside. Among the vast number of studies on B-1 cells, we have carried out a literature review highlighting current trends in the study of B-1 cell involvement during inflammation, which may result in a paradigm shift toward sustainable therapeutics in various inflammatory diseases.

Keywords B-1 cells · Natural IgM · Inflammation · Autoimmunity · Influenza · Sepsis · Atherosclerosis · IBD

Abbreviations

NK	Natural killer
B_{reg}	Regulatory B cells
GM-CSF	Granulocyte–macrophage colony-stimulating factor
IRA	Innate response activator
MZ	Marginal zone
MHC	Major histocompatibility complex
STAT3	Signal transducer and activator of transcription 3
NF-κB	Nuclear factor-κB
PC	Phosphorylcholine
PtC	Phosphatidylcholine
PD-1	Programmed death-1
PD-L1	Programmed death-ligand 1
PD-L2	Programmed death-ligand 2
TLR-4	Toll-like receptor 4
ENPP1	Ectonucleotide pyrophosphatase phosphodiesterase 1
ATP	Adenosine triphosphate
PMA	Phorbol 12-myristate 13-acetate
BCR	B cell receptor
CLP	Cecal ligation and puncture
TNF-α	Tumor necrosis factor-α
Btk	Bruton's tyrosine kinase
OxLDL	Oxidized low-density lipoprotein
Gαi2	G protein α inhibitory subunit 2-deficient
EAE	Experimental autoimmune encephalomyelitis

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EAU Experimental autoimmune uveitis
T1D Type 1 diabetes

Introduction

Inflammation accompanies the body's immune response. An inadequate response may lead to a lack of clearance of invading organisms, whereas a hyperimmune response could cause tissue damage and even death of the host [103]. Immediately after the host encounters a pathogen, the innate immune system consisting of neutrophils, macrophages, dendritic cells, natural killer (NK) cells, and circulating proteins becomes active to confer protection. In contrast, the adaptive immune system which includes antibodies (Abs) produced by B lymphocytes and T cell-mediated immunity becomes responsive at a later time point [103]. Although a large proportion of mature B cells are primarily responsible for mediating adaptive immunity via Ab production, B cells also contribute to innate immunity and exhibit critical immunoregulatory roles. For the purpose of this review, we will focus on B-1a cells unless otherwise mentioned.

In mice, mature B cells consist of three major subsets: (1) follicular B cells, also known as B-2 cells, located in lymphoid follicles, (2) marginal zone (MZ) B cells localized proximal to the marginal sinus of the spleen, and (3) B-1 cells most abundant in peritoneal and pleural cavities [40, 66]. B-2 cells mount Ab responses in a T cell-dependent manner, while both MZ B cells and B-1 cells generate T cell-independent responses [66, 67]. Depending on the presence or absence of surface CD5, a pan T cell marker, B-1 cells can be further subdivided into B-1a ($CD5^+$) and B-1b ($CD5^-$) populations [54]. A growing body of evidence supports considering B-1 cells to be a part of the innate immune system, whereas B-2 cells function primarily in adaptive immune responses [66, 74].

Murine B-1a cells spontaneously secrete and maintain steady-state levels of natural Abs of the immunoglobulin M (IgM) isotype in naïve, non-immunized, and antigen-free mice (reviewed in [34]). B-1a cell-derived natural Abs are found to counteract a wide range of viral and bacterial infections [8]. Recently, a specific role for B-1b cells in generating long-lasting immunity to the relapsing fever bacterium *B. hermsii* has also been demonstrated [2]. This is consistent with other evidence that to combat pathogens B-1a cells secrete natural Abs that protect against infection or lower bacterial burden if infection is established, whereas B-1b cells secrete induced antibody needed to clear certain bacteria and permit survival [1, 36]. The natural Abs secreted from B-1a cells not only neutralize invading pathogens but also recognize and clear dying cells leading to suppression of uncontrolled inflammation and autoimmunity [34].

Soon after the discovery of B-1 cells in mice [40], a number of studies demonstrated their role in various inflammatory diseases. Our current review encompasses the latest trends of B-1 cell pathobiology by revisiting its immunomodulatory functions in terms of natural Ab secretion, antigen presentation, phagocytosis, T cell polarization, and immune suppression in order to help define the therapeutic potential of B-1 cells during inflammation.

B-1 cells: a brief overview

Phenotype and localization

B-1 cells comprise a minor portion of the total B cells in mice and display unique features in terms of their surface phenotype, localization, ontogenesis, and function [36, 40, 41, 54, 62, 74, 97]. The cell surface phenotype of murine B-1 cells is $CD45R(B220)^{lo}$, surface IgM $(sIgM)^{hi}$, $sIgD^{lo}$, $CD23^{lo/-}$, $CD19^{hi}$ and $CD43^+$ and can be either $CD5^+$ (B-1a) or $CD5^-$ (B-1b) [53, 54, 100]. B-1a cells are predominantly localized in the peritoneal cavity, which accounts for a major portion of the total B cells of this compartment. B-1a cells are also found in spleen, pleural cavity, and bone marrow, but are barely detectable in the blood and lymph nodes [40, 53, 60, 114]. Most of the B-1a cells in the peritoneal and pleural cavities express CD11b, a macrophage–granulocyte marker; however, the majority of the B-1a cells in spleen do not express this marker [41, 53].

Ontogeny and development

B-1a cells represent a distinct developmental lineage derived from a unique progenitor found in the fetal liver as well as in fetal and adult bone marrow [75]. The discovery of a B-1 cell-specific progenitor resolved the long-lasting origin debate on lineage versus differentiation concepts (reviewed in [94, 99]). Transfer of the B-1 cell progenitor $(Lineage^-(Lin^-)B220^{lo/-}CD19^+)$ into immunodeficient recipients efficiently reconstituted B-1a and B-1b cells [75]. B-1 cell progenitors do not express syndecan-1 (CD138) or major histocompatibility complex (MHC) class II Ags [101]. B-1 cell progenitors first appear in the fetal liver around day 11 of gestation, at which time no $CD45R^+$ B-2 progenitor cells are observed. Similarly, no $CD45R^+$ cells are observed in fetal bone marrow from embryonic day 15, while the $CD45R^{-/lo}CD19^+$ population is well detected [75].

The development of B-1 cells depends on IL-7Rα and Flt-3 ligand and is negatively regulated by Bruton's tyrosine kinase (Btk) [38, 51]. Recently, it has been shown B-1a cells can also be generated by adult bone marrow [26, 45] and B-1 cell-specific progenitors are found in adult bone marrow [75]. However, the extent to which input

from adult bone marrow into the adult B-1a cell pool occurs is still being investigated. In adulthood, the B-1a cell pool is primarily maintained by self-renewal, in which mature, surface Ig-bearing B-1 cells give rise to their own progeny [38]. Circulating B-2 B cells by contrast generally lack the ability to self-renew and are instead replenished by proliferative cells in the bone marrow [38, 51]. The exclusive ability of B-1a cells to self-renew is supported by the finding that these cells constitutively phosphorylate activated signal transducer and activator of transcription 3 (STAT3), which may play a key role in positively regulating cyclin D2 expression that contributes to the proliferation of these cells [38, 56, 74]. The expansion of B-1a cells has been shown to be controlled at least in part by Siglec-G, which is a cell-inhibiting receptor that inhibits calcium signaling and nuclear factor-κB (NF-κB) activation [43]. In addition to Siglec-G, CD22, another co-inhibitory receptor, has also been shown to inhibit the proliferation of B-1 cells [50]. All together these findings demonstrate that B-1a cells appear early in the life as a developmentally separate and distinct lineage.

B-1 cell function

The main function of B-1a cells in the innate immune system is spontaneous secretion of natural Abs, thereby maintaining resting immunoglobulin levels in the body without any stimulus or immunization [34]. Natural Abs form a preexisting shield against infection providing protection during the period of time required for germinal center formation and production of adaptive Abs. It has been estimated that 80–90 % of resting serum IgM and 50 % of serum IgA are derived from B-1a cells (reviewed in [86]). Natural IgM that accumulates in the serum is produced by B-1 cells residing in the spleen and bone marrow [27, 34]. It has been shown that in animals treated with endotoxin, peritoneal B-1a cells migrate to the spleen where they secrete higher amounts of Abs [35, 58, 112]. The natural Abs produced by B-1a cells differ from B-2 cell adaptive Abs in that they display little or no somatic hypermutation and minimal N-region addition, thus reflecting germline sequences [11, 27]. Murine B-1a cell natural Abs are characteristically repertoire skewed, low affinity, and polyreactive. B-1a cell-derived natural Abs are able to recognize phosphorylcholine (PC), an invariant constituent of Gram-positive microbial membranes such as the *Streptococcus pneumonia* bacterial cell wall, as well as membranes of other bacterial pathogens, apoptotic cell membranes, and oxidized lipids [34, 86]. In addition, natural Abs produced by B-1a cells recognize phosphatidylcholine (PtC), a key constituent of senescent red blood cell membranes [71]. Interaction of such natural Abs with an infectious agent can act either by direct inhibition, complement activation, or opsonization leading to phagocytosis and/or Ab-dependent cell-mediated cytotoxicity [34, 86]. Natural Abs from B-1a cells are often autoreactive and help eliminate dead cells and their debris [57]. In so doing, potentially noxious molecules are removed before tissue injury can occur as a result of uncontrolled immune cell activation.

Apart from natural IgM, B-1a cells also spontaneously secrete IL-10 and, after stimulation with lipopolysaccharides (LPS), secrete GM-CSF and IL-3 [23, 96, 116]. In addition, B-1a cells are efficient antigen-presenting cells, providing effective signaling to T cells via co-stimulatory molecules CD80/CD86, which are constitutively expressed on B-1a cells [34, 86, 118]. Furthermore, B-1a cells are able to induce T cell proliferation and help induce CD4 T cell differentiation to pro-inflammatory Th17 cells [119].

B-1-related cells and subsets in innate immunity

B-1a cells are part of the innate immune system, as noted above. Some functions of B-1a cells are segregated to a greater or lesser extent within particular subsets of B-1a cells defined by phenotypic markers, such as PD-L2, CD73, and PC-1. In addition, several other B cell subsets manifest activities that overlap with B-1a cells, including B10/B_{reg} cells, and IRA B cells.

Regulatory B (B_{reg}) cells/B10 cells

In the early 1990s, it was reported that mouse peritoneal $CD5^+$ B-1a cells are the major source of IL-10, especially in the absence of stimulation, which raised the possibility of these cells having immunoregulatory properties [79]. The term 'regulatory B cells or B_{reg} cells' was coined in 2006 representing a specific subset of B cells with immunoregulatory functions [73]. This B_{reg} cell subset has more recently been labeled B10 cells. B10 cells are able to downregulate innate immune responses [113]. Currently, there are no phenotypic, transcription factors, or lineage markers that are unique to B10 cells, although the majority appear to correspond to B-1 cells. Therefore, B10 cells in mice and humans are functionally defined and enumerated by their competence to express measurable IL-10 following ex vivo stimulation with phorbol 12-myristate 13-acetate (PMA) and ionomycin [96, 109]. B10 cells are predominantly found within the spleen and peritoneal cavity and are also present in small numbers within blood, lymph nodes, Peyer's patches, and intestinal tissues [96, 109, 110]. The generalized ex vivo phenotype of B10 cells from untreated mice is $IgM^{hi}IgD^{lo}CD1d^{hi}CD5^+CD19^{hi}CD23^{lo}B220^{hi}$, with <10 % co-expressing IgG or IgA [68, 96, 109].

Innate response activator B cells

B cells have been shown to produce several cytokines and growth factors both in vitro and in vivo. Recently, Rauch et al. [83] identified a novel B cell population which expresses GM-CSF upon LPS stimulation via the Toll-like receptor 4 (TLR-4) pathway. These cells comprise 1–4 % of the total B cell population in spleen after stimulation and are termed innate response activator (IRA) B cells [83]. In another study, Weber et al. [107] demonstrated the ability of IRA B cells to produce IL-3 if stimulated with LPS, which might play a critical role in emergency myelopoiesis and acute inflammation. Like B-1a cells, IRA B cells are also capable of producing an adequate amount of IgM when stimulated with LPS. The surface phenotype of these cells was identified as $CD19^{+}B220^{+}IgM^{+}MHCII^{+}CD5^{+}CD43^{+}CD93^{+}CD138^{+}VLA4^{+}CD284^{hi}IgD^{+}CD23^{+}CD21^{lo}$ but negative for CD11b, CD3, Ly-6G, Ly-6C, NK1.1, CD49b, Ter119, CD4, CD8, CD11c [83]. After performing adoptive fate mapping and parabiosis experiments using mice lacking B cells and several innate signaling molecules, IRA B cells were shown to be derived from peritoneal B-1a B cells that relocated to the spleen after recognizing LPS with TLR-4 [83]. Transcriptome analysis of IRA B cells and comparison to other B cell subsets revealed that IRA B cells are mainly located in serosal sites, have self-renewal ability, and appear early during embryonic life [83].

PD-L2-expressing B-1a cells

Interaction of the programmed death-1 receptor (PD-1) with its ligand programmed death-ligand 1 (PD-L1) downmodulates lymphocyte activity, implying a role for PD-1 in regulating autoimmunity as well as immune responses to viral and parasitic infections [77, 81]. Like T cells and myeloid cells, B cells also express PD-1, engagement of which by its ligand(s) negatively regulates B cell activity [80]. In studying the PD-1 pathway in B-1a cell-mediated immunity, Zhong et al. [118] found approximately 50–70 % of resting peritoneal B-1a cells that express PD-L2 (CD273, B7-DC), a second PD-1 ligand, which was not present or inducible on conventional B-2 cells. This extended the range of PD-L2 expression and showed that it is constitutively expressed in B cells unlike inducible expression in other cell types. Although $PD\text{-}L2^{+}$ and $PD\text{-}L2^{-}$ B-1a cells are similar in proliferative responses and spontaneous immunoglobulin secretion, $PD\text{-}L2^{+}$ B-1a cells are highly enriched for expression of V_H11 and V_H12 genes and represent the bulk of PtC-binding B-1 cells [117, 118]. Moreover, $PD\text{-}L2^{+}$ B-1a cells are highly enriched for autoreactive specificities. These findings show B-1 cells identified by PD-L2 express a specific repertoire.

CD73-expressing B-1a cells

In a recent study, a novel subset of B-1a cells expressing CD73 and CD39, two ectoenzymes that together catalyze the extracellular dephosphorylation of adenine nucleotides to adenosine, has been identified and shown to play an immunomodulatory role during inflammation [52]. Approximately 30–50 % of total B-1 cells express high levels of CD73, whereas only a very minor portion of conventional B-2 cells express very low levels of CD73 [52]. Although extracellular adenosine triphosphate (ATP) is known to play a proinflammatory role, its dephosphorylated form adenosine is immunosuppressive [39]. Therefore, regulating the balance of extracellular ATP and adenosine, controlled at least in part by $CD73^{+}$ B-1a cells, is important in maintaining immune homeostasis.

PC1-expressing B-1a cells

The two classic functions of B-1a cells, (1) innate like and (2) immunoregulatory, can be segregated between two unique subsets based on the expression of plasma cell alloantigen 1 (PC1), also known as ectonucleotide pyrophosphatase phosphodiesterase 1 (ENPP1), an enzyme involved primarily in the hydrolysis of ATP at the cell surface [104]. These subsets, designated B-1a.$PC1^{lo}$ and B-1a.$PC1^{hi}$, express distinct IgH repertoires and contribute to varied levels of serum and mucosal natural Abs [104]. Adoptively transferred $PC1^{lo}$ cells secreted significantly higher amount of circulating natural IgM and intestinal IgA than $PC1^{hi}$ cells [104]. In contrast, the $PC1^{hi}$ cells produced more IL-10 than $PC1^{lo}$ cells when stimulated with LPS and PMA and were able to regulate Th1 cell differentiation. Furthermore, $PC1^{lo}$ cells generated antigen-specific IgM responses to pneumococcal polysaccharide antigens, whereas $PC1^{hi}$ cells did not. Developmentally, $PC1^{lo}$ cells arise from an early period of B-1a progenitors in fetal life, whereas $PC1^{hi}$ cells are generated at a later time after birth [104]. Therefore, identification of B-1a.$PC1^{lo}$ and B-1a.$PC1^{hi}$ cells extends our understanding of innate immune and immunoregulatory functions mediated by B-1a cells.

Human B-1 cells

The presence of the CD5 antigen on B cells generating autoantibodies in rheumatoid arthritis patients was first considered to be the identifying marker of human B-1 cells [16, 115]. However, CD5 is also variably present on cells of several different human B cell populations, including activated, transitional, and pre-naive B cells, which raised doubts about the phenotypic designation of human B-1 cells in terms of the CD5 antigen [29, 63, 92]. As a result,

confusion regarding the very existence of B-1 or B-1-like cells in the human system remained. Recently, the nature of human B-1 cells was revisited by Griffin et al. who identified a population of B cells in human umbilical cord and peripheral blood that manifested three key functional features of mouse B-1a cells [33]. These functional features include: (1) spontaneous immunoglobulin secretion, (2) constitutive intracellular signaling, and (3) efficient T cell stimulation. This population of human B-1 cells was defined as $CD20^{+}CD27^{+}CD43^{+}$ with little to no surface CD69 and CD70, which are both markedly upregulated after activation of $CD20^{+}CD27^{-}CD43^{-}$ (naive) and $CD20^{+}CD27^{+}CD43^{-}$ (memory) B cells [33, 85]. Identification of human B-1 cells will provide valuable insights for future studies on the role of these cells in human inflammatory diseases. A summary of B-1 cells, B-1 cells subsets, and related B cells, in terms of phenotype, localization, and function is provided in Table 1.

B-1 cells and inflammation

Inflammation refers to the host response to invading pathogens mediated by both the innate and adaptive arms of the immune system. B-1a cells are considered to be part of the innate immune system, whereas B-2 cells primarily act in the adaptive immune system [74]. Recent progress has been made toward elucidation of the role B-1a cells in various inflammatory and autoimmune diseases, which is summarized in this review.

Influenza virus infection

Infection by influenza virus is the most common cause of human respiratory disease [95]. The virus spontaneously manipulates its surface proteins to generate highly variable epitopes, thereby making it difficult to design broadly effective vaccines against influenza virus. The polyreactive nature of B-1a cell-secreted natural Abs enables them to effectively eliminate the influenza virus infection in mice [6, 7]. According to the recent studies, systemic natural IgM secreted by B-1a cells is transported to mucosal surfaces through the poly-Ig receptor leading to protection against influenza virus infection [6, 7]. The importance of systemic natural IgM for protection against influenza virus has been documented in mice deficient in secreted IgM but normal for surface-bound IgM (sIgM), which exhibited reduced clearance of virus and increased mortality following influenza virus infection [7].

Recent findings demonstrate an active role for B-1a cells which provides enhanced local rather than systemic defense against influenza virus [21]. This defense was shown to be mediated through highly localized activation of B-1a cells at lymph nodes close to the respiratory tract, serving as a major source of local virus-neutralizing natural IgM [21]. Surface expression of the CD5 antigen in B-1a cells plays an important role in regulating antigen-driven B cell receptor (BCR)-mediated signaling [93]. During influenza virus infection, $CD5^{+}$ B-1a cell-oriented active responses are not dependent on antigen-induced BCR-mediated proliferation but are mainly generated by their increased accumulation at the lungs and its related lymph nodes as a result of their translocation from serosal cavities where they spontaneously secrete natural Abs and other immunomodulatory molecules to confer protection against influenza virus infection [21, 93]. B-1b or conventional B-2-cells on the other hand provide protection against pathogens through steps involving clonal expansion via BCR-mediated signals in the absence of CD5 receptor on their surfaces [2].

In some cases of infection, B-1a cell-mediated immune regulation may not be beneficial. A recent study revealed Xid mice deficient in B-1a cells showed better protection than normal animals following infection with a virulent strain of *Francisella tularensis* [24]. The poor performance by normal mice could be the result of B-1a cell generated IL-10 creating an immunosuppressive environment in which virulent strains could more easily be replicated rather than destroyed [24], a situation relieved in the absence of B-1a cells. Therefore, a finely tuned balance between B-1a mechanisms promoting immune defense and immune suppression is required for efficient viral clearance and recovery from infection.

Streptococcus pneumonia infection

Streptococcus pneumoniae causes acute bacterial infection especially in the lungs. Approximately 400,000 hospitalizations from pneumococcal pneumonia infection are estimated to occur annually in the USA, leading to 5–7 % case fatality, which is significantly higher among elderly persons [17]. With germline-like IgM and a distinctly skewed repertoire, B-1a cell-derived natural IgM defends against bacterial infections. In particular, anti-PC Ab derived from B-1a cells has been well characterized and shown to be protective against *S. pneumoniae* infection [15, 36, 44]. The importance of anti-PC and anti-pneumococcal capsular polysaccharide 3 (PPS3) germline-like natural Abs was shown in mice lacking B-1a cells, as they were unable to survive acute infection with *S. pneumoniae* [36]. The polyreactive nature of natural IgM sometimes may not be able to effectively neutralize certain invading pathogens. N-addition has been shown to play a key role in antigen receptor diversity and Ab effectiveness to certain pathogens such as *S. pneumoniae*. B-1a cells also secrete the prototypical anti-PC antibody, T15, which has no

Table 1 Phenotype, location and function of B-1 cells, B-1 cell subsets, and B-1-related B cell subsets

Cell types	Phenotype	Location	Function	References
B-1a	$B220^{lo}CD23^{-}CD5^{+}CD19^{+}$ $IgM^{hi}IgD^{lo}CD43^{+}$	PerC, spleen, pleural cavity, bone marrow, intestine	Spontaneously secret polyreactive natural IgM in an antigen-independent manner that recognizes pathogens, OxLDL; promote phagocytic clearance of apoptotic cells; Th17 cell differentiation	[66, 74]
B-1b	$B220^{lo}CD23^{-}CD5^{-}CD19^{+}IgM^{hi}IgD^{lo}$ $CD43^{+}CD1d^{mid}$	PerC, spleen, lung	Produce Abs after encountering T cell-independent antigens	[2]
Marginal zone	$B220^{+}CD23^{-}IgM^{hi}IgD^{lo}CD21^{hi}$ $CD43^{-}CD1d^{hi}$	Marginal sinus of spleen, subcapsular sinus of lymph nodes*	Antigen presentation, elicit T cell-independent immune response	[18, 67]
Follicular	$B220^{hi}CD19^{+}$ $CD23^{hi}CD1d^{mid}CD21^{mid}IgM^{lo}IgD^{hi}$	Germinal centers of spleen	Elicit T cell-dependent responses to protein antigens	[82]
IRA B	$IgM^{hi}IgD^{lo}CD23^{lo}$ $CD43^{+}CD93^{+}$ $VLA4^{hi}CD19^{hi}CD284^{+}GMCSF^{+}$	PerC, spleen, serosal sites	Secrete GM-CSF and regulate IgM production via GM-CSF axis in an autocrine manner; induce emergency myelopoiesis by IL-3 production	[83, 107]
B_{reg}/B10	$CD5^{+}CD19^{hi}$ $CD1d^{+/hi}CD21^{+}CD23^{+/-}$ $CD43^{+/-}IgM^{hi}IgD^{lo}IL10^{+}$	Spleen, PerC, blood*	Regulate inflammatory responses through IL-10 production	[111]
B-1a.PD-L2	$B220^{lo}CD23^{-}CD5^{+}CD173(B7\text{-}DC)^{+}$ $IgM^{hi}IgD^{lo}$	Spleen, PerC	Repertoire-skewed production of PtC-binding V_H11 and V_H12 Ig which play key role in immune defense against bacterial infection	[118]
B-1a.$CD73^{hi}$	$B220^{lo}CD23^{-}CD73^{hi}CD39^{+}$	Spleen, PerC	Produce adenosine in the presence of substrate and play an immunomodulatory role during inflammation	[52]
B-1a.$PC1^{lo/hi}$	$CD19^{+}CD5^{+}CD23^{-}PC1^{lo/hi}$	PerC	Play innate-like or immunoregulatory function based on natural IgM or IL-10 production by PC1 low- and high-expressing B-1a subsets, respectively	[104]
IL-35^{+} B-1	$IgM^{+}CD138^{hi}$ $CD43^{+}TACI^{+}CXCR4^{+}CD1d^{mid}$ $Tim1^{mid}$	Spleen	Express anti-inflammatory cytokines and regulate T cell-mediated hyper immunity	[90, 105]
$CD138^{+}$ B-1a	$B220^{lo}CD23^{-}$ $CD5^{+}CD138^{+}$ $IgM^{hi}IgD^{lo}$	Spleen	Secrete large amounts of natural IgM which are skewed with respect to N-region addition, and some aspects of V_H and J_H utilization	[46]
Human B-1	$CD3^{-}CD19^{+}CD20^{+}$ $CD27^{+}CD43^{+}$ $CD69^{-}CD70^{-}$	Umbilical cord and adult peripheral blood	Spontaneous natural IgM and IL-10 secretion to regulate inflammation	[33, 85]

* Human, *PerC* peritoneal cavity, *IgM* immunoglobulin M, *OxLDL* oxidized low-density lipoprotein, *GM-CSF* granulocyte–macrophage colony-stimulating factor, *PtC* phosphatidylcholine

N-addition and is completely germline [10, 15]. Terminal deoxy transferase (TdT) adds non-templated nucleotides (N-addition) to the V–D and D–J junctions during antibody gene recombination, increasing junctional diversity. Interestingly, in mice overexpressing TdT, vaccination with heat-killed *S. pneumoniae* generated an anti-PC response, but these anti-PC antibodies were not protective against *S. pneumoniae* infection [9]. This study demonstrates that excessive diversity generated by N-addition can be detrimental for microbial protection.

Utilizing various animal models of acute lung infection by direct intratracheal or intranasal delivery of LPS, *E. coli* or *S. pneumoniae*, Weber et al. [106] showed B-1a B cells migrate from the pleural space to the lung parenchyma and secrete GM-CSF and polyreactive IgM to confer protection against infection. Such a migration model has also been proposed by Baumgarth et al. [5] which suggests that body cavity B-1a cells circulate throughout the animal in the steady state. Following infection by pathogens, innate inflammatory signals contribute to behavioral changes of

B-1a cells in the body cavities, and these responder populations migrate to neighboring lymphoid sites [5].

Sepsis

Sepsis is a pervasive human health concern afflicting 3 million people in the USA every year [25, 30]. Injury from sepsis occurs as a result of an uncontrolled inflammatory response [3]. The role of T and B cells in sepsis was first demonstrated in *Rag1*$^{-/-}$ mice, which were shown to succumb more readily to polymicrobial sepsis [47]. Interestingly, repletion of *Rag1*$^{-/-}$ mice with B cells improves survival, demonstrating that B cell function in the absence of T cell-dependent Abs is important for sepsis outcome [59]. An appropriate immune response is necessary to confer protection against sepsis, while the lack of a first line of defense may exacerbate disease pathogenesis. Intriguingly, a recent study demonstrated the lack of B cells in μMT$^{-/-}$ mice resulted in increased mortality, similar to *Rag1*$^{-/-}$ mice [59]. However, T cell-deficient TCRαβ$^{-/-}$ mice did not display increased susceptibility to sepsis-induced mortality when compared to WT mice. These findings suggest an essential role for B cells and innate immune responses in sepsis.

To evaluate the role of natural IgM in the immediate response against sepsis, one of the first studies conducted was in mutant mice deficient in secreted (s)IgM in an acute peritonitis model induced by cecal ligation and puncture (CLP). This study revealed a significant increase in mortality in sIgM-deficient mice as compared to their WT counterparts [13]. This increased susceptibility was associated with a reduced level of tumor necrosis factor-α (TNF-α), a decreased neutrophil infiltration to clear peritoneal bacteria, and elevated levels of endotoxin and proinflammatory cytokines in the circulation. Conversely, resistance to CLP by sIgM-deficient mice was restored by reconstitution with polyclonal IgM from normal mouse serum [13]. The study also revealed the role of antibody specificity, inasmuch as administration of a monoclonal IgM specific to phosphatidylcholine (PtC) had a protective effect, but a monoclonal IgM specific to phosphorylcholine (PC) was not protective. These findings suggest a critical role of natural IgM for immediate defense against severe bacterial infection and thus suggest a role for B-1a cells in responding to sepsis [13]. The consequences of sIgM in the murine sepsis model can be correlated with clinical findings where specific changes in circulating IgM occur when patients with severe sepsis progress into septic shock [32]. In this hospital-based study, paired comparisons at distinct time points over the course of sepsis showed IgM was decreased only when patients deteriorated from severe sepsis to septic shock. Consequently, the study also revealed that production of IgM by B cells was significantly lower at all stages of sepsis compared with healthy controls [32]. Considering altered circulatory IgM levels as one of the fate determining factors for patients with sepsis, a clinical trial was conducted by administration of an IgM-enriched Ig preparation, known as Pentaglobin therapy, monitoring the progression of organ failure and septic shock in patients with severe sepsis [98]. Although like other clinical trials Pentaglobin therapy did not produce an improvement in any of the outcome measures of patients with severe sepsis, it was shown to have promising inhibitory effects on TNF-α production in vitro [98]. Since B-1 cells are known to contribute to the homeostasis of serum IgM levels, assessment of the status of B-1a cells in terms of their frequencies and numbers at different organs may establish a link between decreased serum levels of IgM and B-1a cell contents during sepsis.

The direct role of B-1 cells to temper endotoxemic inflammatory responses has been demonstrated recently by utilizing B-1 cell-deficient Xid mice which showed increased mortality over WT counterparts during endotoxemia [4]. Increased levels of pro-inflammatory cytokines, TNF-α, IL-6 and decreased levels of IL-10 were found in plasma, lung, and gut in Xid mice after sepsis [4]. By utilizing macrophage and B-1a cell co-culture experiments, the investigators demonstrated that the immunomodulatory function of B-1a cells in sepsis could be mediated through IL-10 production [4]. However, apart from IL-10-mediated immune regulation by B-1a cells, other relevant work suggests that the protective role of B-1a cells in sepsis could also be mediated through natural IgM production to neutralize endotoxin [84]. Reconstitution of Bruton's tyrosine kinase (Btk)-deficient mice, which lack B-1a cells and have reduced levels of lgG3 and IgM, with purified normal mouse IgM dramatically enhances their ability to clear endotoxin [84]. The pathogenesis of sepsis may also arise due to uncontrolled accumulation of apoptotic cells in organs; it is therefore speculated that B-1 cell natural IgM could facilitate the clearance of apoptotic cells by phagocytes, which might aid in the amelioration of sepsis.

In response to LPS stimulation, peritoneal B-1a cells proliferate, migrate to the spleen, and give rise to the GM-CSF^{+} IRA B cell population [83]. In a murine model of sepsis, mice with a B cell-restricted deficiency in GM-CSF showed increased neutrophil infiltration to the peritoneum [83]. However, these neutrophils had impaired phagocytic activity, and the mice experienced a severe cytokine storm and died. This suggests that GM-CSF-producing B cells contribute to bacterial clearance by promoting neutrophil phagocytic function [83]. In contrast to identification of a beneficial role of IRA B cells in sepsis, a recent report also revealed that these cells produce IL-3, which might degrade sepsis outcomes [107]. IL-3 production by these cells enhanced emergency myelopoiesis leading to

uncontrolled cytokine storm during sepsis [107]. By contrast, although IL-3 production by IRA B cells was shown to be detrimental in systemic inflammation due to accelerated emergency myelopoiesis, a recent study revealed that improved emergency myelopoiesis could be beneficial for survival in neonatal sepsis [31].

Atherosclerosis

Atherosclerosis is a chronic inflammatory disorder, in which leukocytes infiltrated within the atherosclerotic vessels augment inflammation by increasing the expression of adhesion molecules, cytokines, matrix metalloproteinases, and tissue factor [102]. Inflammation during atherosclerosis is associated with increased levels of oxidized low-density lipoproteins (OxLDL) trapped in the arterial walls [65]. B-1a cells have been shown to be atheroprotective by their production of natural IgM that eliminates OxLDL as well as apoptotic and necrotic cells [61]. Healthy individuals contain basal levels of natural IgM which recognize OxLDL [72]. It has been reported that when atherosclerosis-prone mice were injected with heat-killed *S. pneumonia*, they generated high levels of serum anti-OxLDL IgM that protects against atherosclerosis [12]. The direct role of natural IgM in atherosclerosis has also been shown by administration of natural IgM to mice with advanced atherosclerosis, leading to decreased severity of the disease [19]. Conversely, mice deficient in secretory IgM or C1q developed increased atherosclerotic lesions as compared to mice with normal levels of serum IgM or C1q (reviewed in [27]). Since B-1a cells maintain overall resting serum IgM levels, their role in protection against atherosclerosis can easily be understood. In support of the above statement, recent literature reviews also indicate that B-1a cells and B-1a cell-derived natural IgM are protective against atherosclerosis [61]. However, in addition to natural IgM, B-1a cells may also spontaneously secrete IL-10 and adenosine, which inhibit inflammation produced by activated macrophages and T cells present in atherosclerotic lesions [61]. Moreover, the B-1a cells can undergo in vivo expansion induced by self-antigens or IL-9 administration, which could serve as a potential therapeutic strategy to inhibit atherosclerosis (reviewed in [61]).

Beyond reports that suggest natural antibodies, IL-10, and adenosine produced by B-1a and B10 cells may counteract inflammation that is part of atherosclerotic disease, a recent study demonstrated that GM-CSF secreting IRA B cells did not show any protection in atherosclerosis [42]. In response to a high-cholesterol diet, IRA B cell numbers in mice and humans increased preferentially in secondary lymphoid organs via Myd88-dependent signaling [42]. However, this increase may contribute to pathology inasmuch as mixed chimera mice lacking B cell-derived GM-CSF developed smaller lesions with fewer macrophages and effector T cells. Mechanistically, IRA B cells promote the expansion of classical dendritic cells, which then generate IFN-γ-producing Th1 cells. This IRA B cell-dependent Th1 skewing manifests in an IgG1 to IgG2c isotype switch in the immunoglobulin response against oxidized lipoproteins. Thus, the IRA B cells alter adaptive immune processes and shift leukocyte responses toward a Th1-associated milieu that aggravates atherosclerosis [42]. However, since these IRA B cells still secrete natural IgM which could be beneficial, their role in atherosclerosis needs to be further evaluated.

Inflammatory bowel disease

Inflammatory bowel disease (IBD), along with its two clinical subtypes, Crohn's disease (CD) and ulcerative colitis (UC) is characterized by chronic and relapsing forms of severe gastrointestinal tract inflammation [49]. In the USA, it is currently estimated that about 1–1.3 million people suffer from IBD [55]. The pivotal immunoregulatory role of B-1a cells in protection against colitis has recently been shown by utilizing TCRα$^{-/-}$ mice [91]. Under specific pathogen-free (SPF) conditions, TCRα$^{-/-}$ mice were found to be more susceptible to develop colitis than similar animals in a conventional facility [91]. Of note, the mice kept in the conventional facility had increased levels of natural IgM produced by B-1a cells to suppress colitis initiation. Conversely, B cell-deficient Igμ (Igh6) and TCRα double-knockout (αμDKO) mice housed in a conventional facility continued to develop severe colitis, suggesting a protective role for B-1a cells. Further, when these αμDKO mice within the conventional facility were adoptively transferred with peritoneal B-1a cells isolated from TCRα$^{-/-}$ mice they became protected against colitis [91].

IL-10 is known to play an immunoregulatory role during colitis, as mice deficient in IL-10 develop spontaneous colitis with many similarities to human Crohn's disease [64]. The therapeutic potential of IL-10 producing regulatory B cells or B10 cells in experimental colitis has been demonstrated in recent reports. Studies in CD19$^{-/-}$ mice, which have few, if any B10 cells, revealed more severe dextran sulfate sodium-induced intestinal injury than in WT animals [111]. Remarkably, adoptive transfer of B10 cells isolated from the spleens of WT mice reduced inflammation in CD19$^{-/-}$ mice by improving the physiological, histological, and immunological parameters in an IL-10-dependent manner, which may provide new insights and therapeutic approaches for treating ulcerative colitis [111]. Consistent with findings in the CD19$^{-/-}$ mice, G protein α inhibitory subunit 2 knockout (Gαi2$^{-/-}$) mice which have significantly reduced numbers of B-1a, B10,

MZ, and T2 B cells can spontaneously develop IBD [87]. Besides the protective role of splenic B10 cells in colitis, a recent report characterized peritoneal cavity B10 cells in terms of spontaneous or inducible IL-10 production and validated their function in colitis [69]. Importantly, these IL-10 producing peritoneal cavity B cells significantly reduced disease severity in spontaneous and induced models of colitis by regulating neutrophil infiltration, colitogenic $CD4^+$ T cell activation, and proinflammatory cytokine production during the onset of colitis, thereby helping to maintain homeostasis within gastrointestinal tissues and the immune system. A recent report regarding adenosine-producing CD73 expressing B-1a cells showed protection against chemically induced colitis in mice [52]. Collectively, these studies clearly demonstrate the potential therapeutic role of B-1a, B10, and B_{reg} cells during intestinal inflammation via IgM, IL-10, and adenosine production.

Autoimmune diseases

Autoimmune diseases can affect almost any part of the body and typically have a major inflammatory component [76]. Different functions of B cells, especially the secretion of autoantibodies, presentation of autoantigens, and secretion of inflammatory cytokines contribute to autoimmune diseases [37]. Considering the ability of B-1a cells to present antigen and skew T cells toward Th17 differentiation, they may promote autoimmunity if their immunosuppressive functions as mediated by IL-10 and adenosine production become dysfunctional [86, 88]. The autoreactive nature of natural IgM speeds the elimination of dead and dying cells and cellular debris [14]. By contrast, mice lacking natural IgM are prone to accelerated development of IgG autoantibodies and more severe autoimmune diseases, presumably because antigens and inflammation associated with apoptotic cell debris stimulate B-2 cell responses when not properly cleared in a timely fashion [14].

The importance of IL-10 producing regulatory B cells for controlling experimental autoimmune encephalomyelitis (EAE), a Th1-mediated autoimmune disease of the central nervous system (CNS), has been reported recently [28, 70]. The role of B-1a cells as well as IL-10 producing regulatory B cells in controlling the development of EAE has been examined in $CD19^{-/-}$ mice, which are deficient in B-1a cells [70]. The study showed that the greater severity of EAE in $CD19^{-/-}$ mice as compared to WT animals was associated with polarized Th1 cytokines in the inflamed CNS, causing exaggerated neuroinflammation [70].

Interleukin-35 belongs to the IL-12 family of cytokines and represents a potential therapeutic target in autoimmune, inflammatory, and infectious diseases. The immunosuppressive effect of IL-35 is mediated through regulatory T and B cells [22]. Wang et al. [105] recently showed that IL-35 induced B_{reg} cells to secrete both IL-10 and IL-35. Injection of recombinant IL-35 or $IL\text{-}35^+$ B_{reg} cells into mice after induction of experimental autoimmune uveitis (EAU), a mouse model of autoimmune eye disease, resolved inflammation by suppressing effector Th1 and Th17 cell responses and inducing $Foxp3^+$ T_{reg} cells [105]. Besides the work on $IL\text{-}35^+$ B_{reg} cell-mediated protection against EAU, a supportive study by Shen et al. [90] identified TLR-4 and CD40L co-stimulation as the main driver of B cell-mediated IL-35 production in a mouse model of EAE. The importance of $IL\text{-}35^+$ B_{reg} cells was demonstrated in mice lacking B cell-specific IL-35, which resulted in substantially worse outcomes in EAE [90]. B cells lacking IL-35 expressed higher levels of co-stimulatory molecules and functioned as more potent antigen-presenting cells to T cells compared to IL-35 expressing B cells, leading to stronger inflammatory T cell responses [90]. Beyond IL-35, a recent study demonstrated that $PD\text{-}L2^+$ B-1a cells may play a role in opposing the development of autoimmunity not only by PD-1/PD-L2-mediated immunosuppression but also by their specific natural IgM production [118]. Collectively, these features reveal the importance of B-1a and B_{reg} cells for protection against autoimmune diseases by secretion of immunoregulatory molecules.

Obesity and diabetes mellitus

Obesity is associated with a state of chronic, but low-grade inflammation [108]. IL-10-secreting regulatory B cells have an anti-inflammatory role in murine obesity [89]. Nishimura et al. [78] recently identified a distinct set of IL-10 secreting B cells, which negatively regulate adipose tissue inflammation. The phenotype of these IL-10 secreting B cells from subcutaneous and epididymal adipose tissue was identified as $CD1d^{lo}CD5^{-/lo}CD11b^{lo}CD21/CD35^{lo}CD23^{-/lo}CD25^+CD69^+CD72^{hi}CD185^-CD196^+IgM^+IgD^+$, which was phenotypically distinct from other IL-10 secreting B cells isolated from spleen and peritoneal cavity expressing $CD1d^{hi}CD5^+$ [78]. It has been shown that with a deficiency of these distinct IL-10 producing B cells, infiltration of pathogenic T cells and macrophages in adipose tissue of obese mice was higher than in control animals [82]. In addition, obesity is closely linked to other metabolic diseases such as insulin resistance and type 2 diabetes mellitus where regulatory B cells play crucial roles [78]. Studies using lean mice lacking B_{reg} cells exhibited insulin resistance and impaired fasting glucose clearance [82]. A recent study revealed that B cells secrete a short peptide derived from proteolytic cleavage of adiponectin, which negatively regulates T cell trafficking across inflamed endothelium [20]. Interestingly, patients with type 1 diabetes (T1D) had reduced serum levels of the adiponectin-derived short

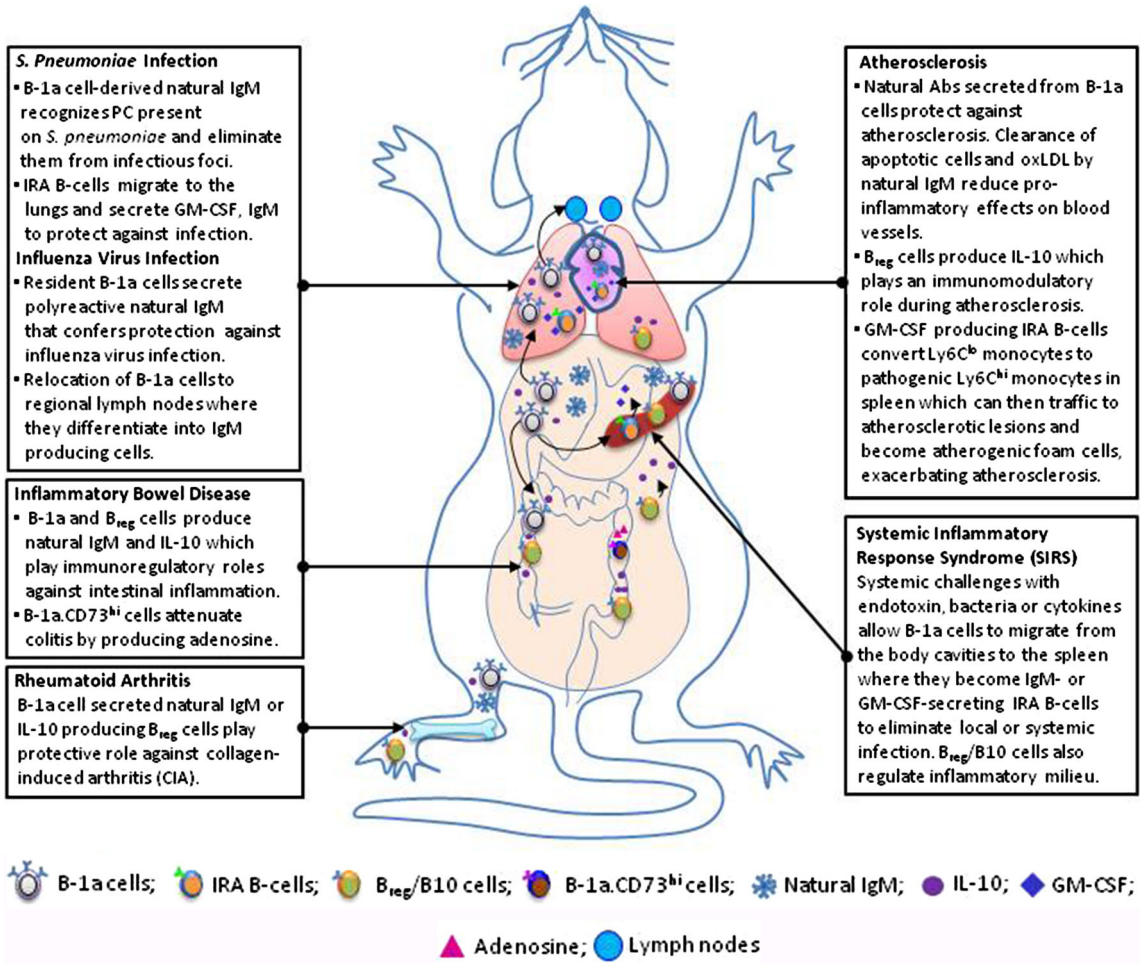

Fig. 1 Role of B-1 cells in inflammatory diseases. B-1 cells recognize endogenous antigens and invading pathogens through B cell receptor (BCR) or Toll-like receptor (TLR)-mediated pathway. Reorganization of Ag causes B-1a cells to migrate from the peritoneal or pleural cavities to the regional lymph nodes or spleen where they may become activated to IgM producing B cells/memory B cells. The polyreactive, repertoire-skewed natural IgM recognize wide range of endogenous and exogenous Ag to eliminate from the host, thus reducing systemic inflammation. GM-CSF secreting IRA B cells migrate to the spleen and lungs and confer protection against systemic as well as local *S. pneumonia*-induced inflammation. By contrast, the IRA B cells convert $Ly6C^{lo}$ monocytes to pathogenic $Ly6C^{hi}$ monocytes in the spleen which then traffic to the atherosclerotic lesions and become atherogenic foam cells, thereby exacerbating atherosclerosis. Apart from B-1 and IRA B cells, regulatory B cells play protective roles against various systemic, autoimmune, and inflammatory bowel disease by producing immunoregulatory cytokine, IL-10, and natural IgM

peptide, which implicates B cells in controlling T1D pathogenesis [20]. Moreover, protection from T1D requires B cell-derived IL-10 production, because transfusion of activated NOD-IL-$10^{-/-}$ B cells does not confer protection from T1D in NOD recipients [48]. Thus, evaluation of B-1a cells as well as B_{reg} cells in various inflammatory diseases is likely to provide potential therapeutic possibilities (Fig. 1).

Conclusions and perspectives

Three decades of research on B-1 cells along with related B cells and subsets demonstrate that B-1 cells are indispensable for protection against various inflammatory diseases and for maintaining normal homeostasis. Although ample studies have been carried out to characterize phenotypic, ontogenic, and functional profiles, additional work is still required to understand the complexity of how B-1 cells are involved in host–pathogen interactions in various inflammatory diseases. The role of B-1 cells in cerebral, intestinal, or hepatic ischemia and other systemic inflammatory diseases is yet to be evaluated and could open new fields of research. Apart from natural IgM, current studies also focus on identifying novel molecules that are being secreted by B-1 cells to modulate the inflammatory milieu. Further study of novel cellular interactions involving B-1 cells and B-1 cell trafficking in various organs after inflammation may resolve several

unanswered questions of disease pathogenesis. A clear dissection of B-1 cell biology will help to develop novel approaches for the prevention of infectious, autoimmune, and inflammatory diseases.

Acknowledgments This study was supported by the National Institutes of Health (NIH) Grants R01GM053008 and R01GM057468 to PW and R01AI029690 to TR.

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