Chapter 8

B Lymphocyte as a Target of Bacterial Infections

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Abstract

B lymphocytes are central players in the immune response; canonically, they have been recognized as precursors of antibody-producing cells: plasma cells. Recent findings have shown that the role of B lymphocytes goes far beyond the production of antibodies. There are different subtypes of B lymphocytes with different participations in innate and adaptive responses that include the recognition of the antigen, its processing, and its presentation to T lymphocytes, as well as the production of cytokines that impact and modulate the response toward the pathogen. Traditionally, it has been considered that B lymphocytes do not have phagocytic abilities that allow them to internalize, to process, or even to be infected by bacterial pathogens. The new information has shown that B lymphocytes can be readily infected by bacterial pathogens like *Salmonella*, *Francisella*, *Moraxella*, and *Mycobacterium*, among others, and respond to those infections. Some of the recent advances on these topics will be presented in this chapter.

Keywords: B lymphocyte, B1 lymphocytes, bacterial infection, *Brucella*, *Salmonella*, *Mycobacterium*, *Francisella*, endocytosis, macropinocytosis, innate response

1. Introduction

1.1. A brief history on the B-cell discovery

The immune response comprises cellular and humoral elements; among the cellular elements, macrophages were the first cells to be described by Metchnikoff in the year 1889 [1]. Almost simultaneously with Metchnikoff findings, began the recognition of one of the primordial

elements of the specific humoral response: the antibodies. Von Bering and Kitasato in 1880 present their first work on serotherapy, showing that in the serum of animals immunized with diphtheria or tetanus toxins, there were specific elements of recognition for these toxins and that their use in patients conferred protection [2]. Later, Phlizalix and Calmette groups independently produce antisera against snake venoms [3], confirming the relevance of specific serum elements for the protective response. In those days, histological studies on organs of experimental animals subjected to immunization processes suggested that lymphoid organs were sites likely responsible for the synthesis of the serum elements responsible for protection against toxins and poisons [4]. Tisselius and Kabat in the 1930s demonstrated by electrophoretic techniques that humoral elements responsible for the serological protective response against toxins and poisons belong to the serum gamma globulin fraction [5]. Some years later, in the 1940s, ex vivo culture of plasma cells from the spleen of hyperimmune animals was achieved, and it was observed that, even in culture, the plasma cells were still producing the specific antibodies [6]. Later, the development and application of fluorescence techniques allowed the identification of antibodies in situ, locating them closely with the plasma cells present in secondary lymphoid organs [4]. Nossal´s experiments demonstrated that antibodies produced by plasma cells isolated from immunized animals retained biological activity in vitro against the bacteria used in the immunization [7]. Human immunodeficiency studies, like Bruton's immunodeficiency, which is characterized by the absence of gamma globulin production and the absence of plasma cells in lymphoid organs [8, 9], demonstrated now in humans that antibodies were produced by plasma cells. The Landstainer and Chase experiments excluded antibodies as mediators of cell-mediated hypersensitivity responses [10], and it is in the 1960s when observations on the development of the immune response in thymectomized and reconstituted animals allowed to recognize the thymus as a fundamental organ of the immune response responsible for grafts rejection but also contributed to antibody production [11, 12]. The bird model for study of elements of the immune response initiated the identification of the organ responsible for B-lymphocyte production [13], and in 1965, in birds, it was demonstrated that in the thymus and in the bursa, the two cellular lineages fundamental for the immune response are generated [14]. Almost simultaneously, and thanks to the use of radioactive labels, it was demonstrated that circulating lymphocytes stimulated with antigen were the precursors of antibody-producing cells [15, 16], and by the year 1969, the two populations of lymphocytes were identified as T lymphocytes for those thymic-dependent, and those thymic-independent (bursa-equivalent) were referred as B lymphocytes [17]. Afterward, it was established in non-avian experimental models that a cooperative response of both lymphocyte species (T and B) was necessary for specific antibody production [18]; these observations prompted a large number of studies that recognized the complexity of T-B cooperation, resulting in specific responses to the antigen, including the production of specific high-affinity antibodies [19].

1.2. B lymphocytes: a bridge between innate and adaptive immune responses

It is now known that there are several types of B lymphocytes [20] and that B2 lymphocytes produce specific antibodies during the adaptive response [21]. On the other side, there are also natural antibodies of IgM class mainly [22]; unlike the adaptive antibodies, the natural

antibodies are produced by B1 lymphocytes [23]. B1 lymphocytes are subdivided into B1-a and B1-b—subtype B1-a is responsible for natural antibody production—respond to T-independent antigenic challenges, are located mainly in the peritoneal and pleural cavity, and represent the first line of defense against microbial challenges [24]. In addition, B1-a lymphocytes may internalize and eliminate bacterial pathogens and have CD11b marker [25], resembling a macrophage phenotype. Some authors have suggested that the B1-a subset of lymphocytes and macrophages share lineage relationships, so these B/macrophage bi-phenotypic cells may represent an ancient B-lymphocyte lineage capable of adapting to bacterial challenges and innate responses [26]. The mammalian B1-a subset of lymphocytes could be evolutionary related to B cells from fishes, particularly teleost fishes like rainbow trout, catfish, etc.; circulating B cells in teleost fishes are morphologically similar to mammalian B lymphocytes; they also secrete and express immunoglobulin molecules at the membrane level with IgM, IgD, or IgT/Z isotypes and also possess phagocytic abilities [26]; this evolutionary theory of B cell supports the idea of an innate role of B1-a lymphocytes. In this context, B1-a lymphocytes and natural antibodies represent a bridge between innate and adaptive immunity [27].

2. B-cell subtypes

An important role of B lymphocytes in defense against pathogens is that B cells are part of a long-lived lymphocyte group that participates in the immune response by capturing and concentrating antigens for the presentation and production of antibodies. From the time that a B lineage cell becomes a mature B cell expressing the B-cell receptor (BCR) on its membrane, several transition steps have to occur [20]. During these steps, B cells are directed for negative and probably positive selection involved in generating a mature B-cell repertoire [28]. B lymphocytes have been divided into two subtypes, according to the origin of their development into B1 and B2 cells. B1 lymphocytes are the first cells produced in the ontogeny; in mice, B1 cells are produced in the fetus and are derived from distinct precursors. B1 cells are different from B2 lymphocytes by their capacity of spontaneous antibody production, self-renewal, impossibility for clonal expansion, and low somatic hypermutation [29].

B2 cell precursors in the bone marrow give origin to B-cell populations of the marginal zone and the follicular zone; these B cells are the main populations that respond to antigen contact then forming the germinal centers and therefore are long-term responders. These cell populations are the majority B lymphocytes in the host and are predominant in all lymphoid tissues. Marginal zone (MZ) B lymphocytes in the mouse are restricted to the splenic marginal zone, while their human counterparts appear to be also in blood circulation. In the mouse B1 cells are divided in two subsets, B1-a and B1-b, based on their expression of CD5; B1a cells (CD5+) and B1-b cells (CD5−) appear to share developmental precursors. Apart from the differential expression of CD5, they are phenotypically similar, with few functional differences like the ability for internalizing bacterial pathogens [24, 30].

The main function of B1 lymphocytes is the production of large amounts of natural antibodies of the IgM isotype that responds to encapsulated bacterial infections (among others bacterial

challenges) and the production of IgA associated with mucosal defense against parasites. However, these cells are also able to produce IgG2 and IgG3 isotypes spontaneously, and under certain conditions, they may produce IgE. The production of antibodies by B1 lymphocytes is characterized by being spontaneous although it can be induced by T-cell-independent antigens and certain cytokines [31]. B1 lymphocytes predominate in fetal life but decrease with increasing age and have been reported to increase again in advanced age in mice as in humans. However, in the elderly, despite the increase, these cells are not fully functional, making older guests susceptible to acquire frequent lung infections associated with pneumonia. The natural antibodies have low specificity, so they are able to recognize self-antigens; for this, B1 cells have been identified as potential participants in the development of autoimmune diseases. A high number of B1-a cells have been associated with autoimmunity in human and mouse models. In addition, a greater number of B1 cells have been reported in patients with systemic lupus erythematosus, Sjögren's syndrome, and rheumatoid arthritis [32, 33].

B1 cells are the main population of B lymphocytes that are located in cavities such as the pleura and peritoneum (35–70%); these cells are mobilized between both cavities through the omentum, a process that requires the expression of the chemokine ligand CXCL13. In response to the pathogens, B1 cells are mobilized from their primary location within the peritoneum or pleura to secondary lymphoid organs such as the spleen and lymph nodes and at these sites begin to secrete IgM antibodies, so B1 lymphocytes represent a quick innate response toward bacterial challenges. B1 cells have been reported to be found in the spleen (1–2%), lymphoid nodes (0.1–0.3%), bone marrow (0.1–0.2%), lung parenchyma (0.4–0.6%), intestinal lamina propria (up to 50% are IgA + B cells), and blood (0.3–0.5%). In the peritoneal cavity, a subtype of B1 lymphocytes tend to lose expression of the CD43 molecule, but most of these cells in other tissues retain this marker; however, when B1 cells are activated, they overexpress CD43. In the peritoneal and pleura cavity, most of the B1-a and B1-b lymphocytes express the integrin CD11b but when these cells migrate to other organs such as the spleen downregulate its expression. In terms of functionality, B1 lymphocytes and marginal zone B cells are very similar; for this reason, the subgroup of marginal zone B cells (MZ) has been included in the group of "innate" type B cells, conformed then by B1-a cells, B1-b cells, and B cells of the marginal zone. B-cell MZ is also considered as B regulatory cell since after activation, it produces high levels of IL-10 (**Figure 1**) [34].

B1 lymphocytes in addition to producing the natural antibodies also actively contribute to the bacteria-induced immune response; several groups have explored B1 cell responses to pathogens like *Streptococcus pneumoniae*, *Salmonella* spp., *Francisella* spp., *Borrelia hermsii*, and influenza virus, among others. The antibody response analyzed for each case showed an increase of IgM produced by B1 cells in the spleen, regional lymph nodes, or serum. Some studies support the idea of a heterogeneity of B1 cells, but the causes of this heterogeneity are largely unknown and poorly explored. However, Baumgarth has considered three factors that can modulate the functions of B1 cells: (1) the multiple origin of B1 cells, (2) tissue-specific signals, and (3) differences in exposure and responsiveness of B1 cells toward self and foreign antigens. It has been suggested that it is important to determine the impact of these signals on the functionality of B1 cells, which could clarify much of the biology of this cell population and one of its most important products, the natural IgM [35].

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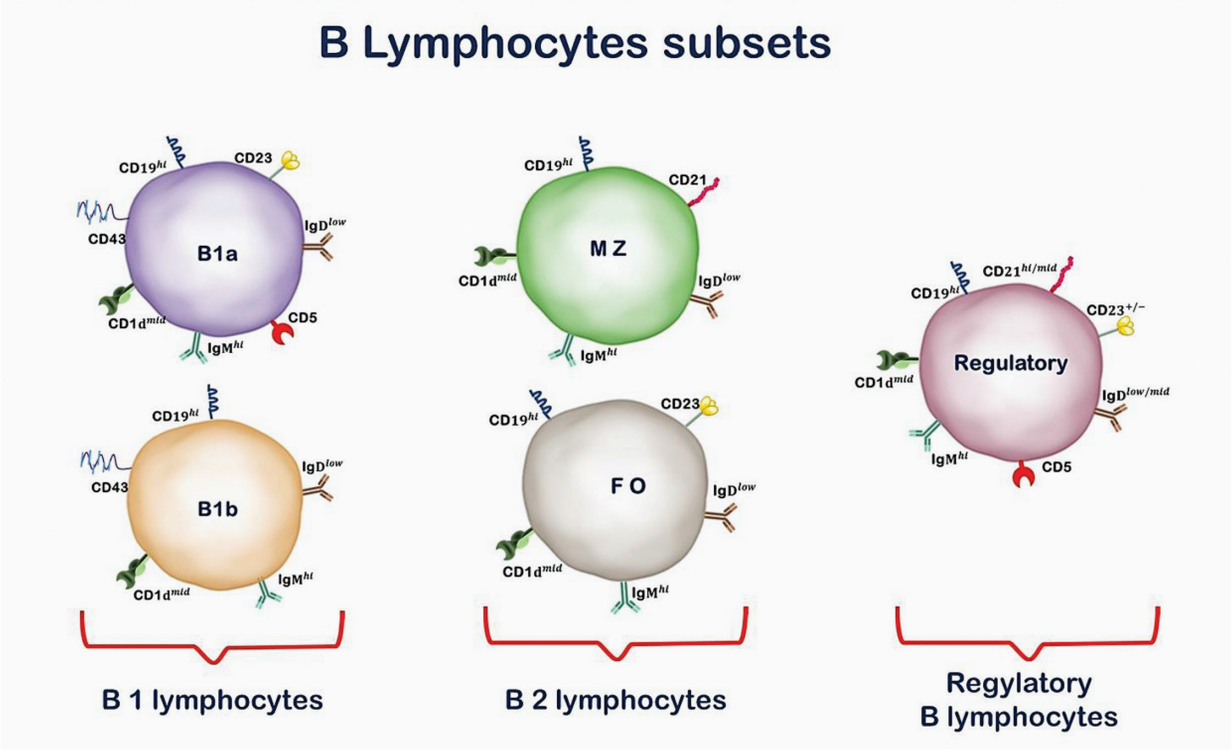

Figure 1. Immunophenotype of mature B-cell subpopulations. The B2-cell population constitutes the majority of spleen B cells formed by follicular cells (FZ) and marginal zone B cells (MZ). B1-a and B1-b cells are smaller populations in terms of frequency in the spleen; they can be distinguished based on CD5 expression: B1-a (CD5+) cells and B1-b (CD5−) cells. It appears that regulatory B cells have phenotypic markers of B1 and B2 cells.

3. B-cell receptors involved in bacterial recognition and uptake

As referred earlier B lymphocytes are not only plasma cell precursors but a heterogenic subset of cells with the capability to act as antigen-presenting cells (PCA) and produce pro- and anti-inflammatory cytokines. Some B-lymphocyte subsets are able, at different rates, to engulf several pathogens predominantly virus and bacteria; this event is mainly mediated by BCR, Toll-like receptors (TLR), and complement receptors (**Figure 2**). In some cases, after bacterial uptake, B lymphocytes are activated and settle a protective or suppressive immune response; also, they may act as pathogen niches or reservoirs that allow bacteria dissemination in the organism. The endocytic pathways developed by B lymphocytes that allow bacterial uptake depend on the receptors engaged during bacterial recognition. In other cases, bacterial components itself trigger in the host cell, mechanisms that allow their entrance into the B cells (**Figure 3**).We will describe some of these elements.

3.1. B-cell receptors

The B-cell receptor (BCR) for antigen is a complex of membrane immunoglobulin (mIg) of isotype IgM^{hi} and IGD^{low} in B1 and B2 lymphocytes and IgM^{hi} and $IgD^{low/mid}$ in B regulatory cells; this complex is responsible for extracellular antigen attachment and is linked to at least two other proteins, Ig α and Ig β forming a heterodimer. The mIg itself does not contain any

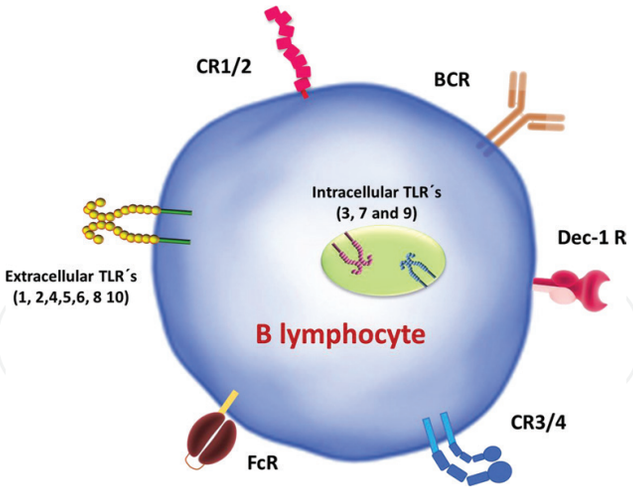

Figure 2. B-lymphocyte receptors involved in pathogens uptake. B lymphocytes displayed a wide number of receptors capable to recognize and engulf pathogens; they include intra- and extracellular innate receptors like TLRs, Dec-1, complement receptors, and the adaptive BCR receptor.

signaling motifs, but instead the Igα/β heterodimer contains immunoreceptor tyrosine-based activation motifs (ITAMs) responsible for initiating the signaling after antigen binding [36]. Membrane Ig (mIg) differs from circulatory antibody in the C-terminus of the heavy chain [37]. B-cell activation is triggered by BCR-antigen interaction and leads to multiple cellular events as BCR-antigen complex internalization, the signalosome assembly, regulation of gene

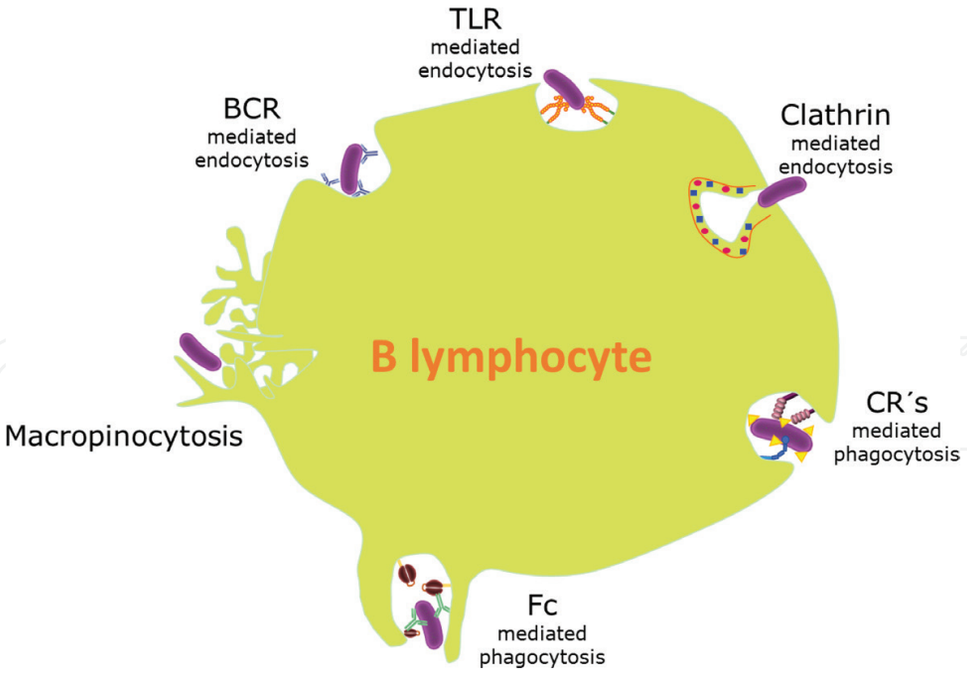

Figure 3. Endocytic pathways in B lymphocytes responsible for bacterial uptake. B lymphocytes are not only precursors of plasma cells but also a multitask cells. Several endocytic pathways may take place on the B cells to internalize bacterial pathogens; the cartoon depicts some of the pathways already described: clathrin-mediated endocytosis, Fc-mediated phagocytosis, CR (complement receptor)-mediated endocytosis, BCR-mediated endocytosis, TLR-mediated endocytosis, and macropinocytosis.

expression, cytoskeleton reorganization, and plasma and long-live B memory cell generation. Antigens internalized through BCR are processed and presented on the major histocompatibility complex II (MHC II). The idea that dimeric BCR complex binding the antigen was enough to activate B cells was accepted for years; nevertheless, it is known that B cells can form microclusters required for B-cell activation [38, 39], which involved around 10–100 BCR molecules; even in this microcluster, BCR complexes can interact in an oligomeric pattern [40]. Most of the studies related to antigen recognition by BCR have been related to soluble antigens [41]; however, more evidences have demonstrated that BCR also recognizes complete pathogens, like *Moraxella catarrhalis* [42] and more recently *Salmonella typhimurium* [43].

3.2. Toll-like receptors (TLRs)

Toll-like receptors (TLRs) are a family of transmembrane and cytoplasmic receptors that are phylogenetically ancient that share homology with the IL-1 receptor; TLRs are part of the group of molecules responsible of pathogen´s recognition that collectively receive the name of pathogen recognition receptors (PRRs) and are expressed in dendritic cells, macrophages, and NK cells (innate immune cells); B and T lymphocytes (cells of the adaptive immunity); and epithelial cells, endothelial cells, and fibroblasts (nonimmune cells). TLRs are responsible for activating the innate immune response [44]. The ligands of the TLRs, known as pathogen-associated molecular patterns (PAMPs), are highly conserved microbial molecules or harmful endogenous factors [damage-associated molecular patterns (DAMPs)]. When B cells-TLRs bound to their ligands, several activation pathways are engaged; two of the most studied are TLR4 and TLR9 activation pathways; TLR4 is expressed on the B-cell membrane along with MD-2 molecule, and this heterodimer participates in lipopolysaccharide (LPS) recognition to initiate several intracellular signaling pathways; one of the most important is the TIRAP-MyD88 pathway that regulates NF-κB activation and inflammatory cytokine production like IL-8, transforming growth factor alpha (TNF-α), etc. TLR9 is another important B cell-TLR, which is expressed in the endoplasmic reticulum and is recruited to endosomal/lysosomal compartments after stimulation with CpG DNAs, activating the MyD88 pathway without TIRAP, culminating in NF-κB activation, and resulting in the production of proinflammatory cytokines [45]. TLRs are classified based on their localization as cell surface TLRs (TLR1, TLR2, TLR4, TLR5, TLR6, and TLR10) or intracellular TLRs (TLR3, TLR7, TLR8, TLR9, TLR11, TLR12, and TLR13); on humans 10 members of the TLR family have been described, while murine cells express 13 TLRs [44]. B lymphocytes express ten types of TLR (TLR1-10) [46–48]; their expression depends on B-cell tissue localization and the stage of B-cell activation. TLRs 3, 4, and 5 and 8 are absent in naive and memory B cells but are present in plasma cells; TLRs 6–9 are highly expressed in naïve B cells, but when they are activated, the intracellular TLR9 is the most highly expressed [45, 49]. Recently, some authors have demonstrated that TLR ligands have a modulatory effect on the B-lymphocyte response, for instance, lipopolysaccharide (LPS) and CpG-containing DNAs promote proliferation, class switching, and plasma differentiation and are directly related with Th1 responses and autoimmune diseases [50–53]. Deficiencies in the downstream signaling pathways in B lymphocytes after TLRs activation favored pathogen infections specially the ones caused by *S. pneumoniae*, *S. aureus*, and *P. aeruginosa* [54]. TLR activation could be also detrimental for the B cells; for instance, B-lymphocyte TLR-2 interacting with *Shigella flexneri* promotes apoptosis of the host cell [55].

3.3. Dectin-1

Dectin-1 (Dec-1) is a transmembrane C-type lectin-like receptor (CTLR) that binds β-glucans; it is expressed mainly in myeloid cells; when Dec-1 recognizes its ligand, the interaction induces B-cell activation with the participation of SYK, the MAPK ERK and JNK, and the transcription factors AP-1 and NF-κB, leading to the production of proinflammatory cytokine production (like IL-8) and arachidonic metabolites synthesis [56, 57]. Dec-1 binds to numerous pathogens through their specific ligands β-1,3 glucans, most frequent in fungi pathogens like *Aspergillus*, *Candida*, *Coccidioides*, and *Pneumocystis* and in nonpathogenic *Penicillium* and *Saccharomyces*; beside Dec-1 can recognize mycobacteria [58]. Dec-1 was first described in dendritic cells; however, now it is known that Dec-1 is expressed in monocytes and lymphocytes. In B lymphocytes, Dec-1 activation leads to IL-8 production and neutrophil chemotaxis which shows that B lymphocytes can directly recognized pathogens via PRRs and have an important role in antifungal response [59].

3.4. Complement receptors

The complement system involves numerous plasma proteins that react with one another, in a cascade-like process that results in molecules that opsonize pathogens and promote inflammatory responses that help to fight infection. Complement proteins execute their biological functions by binding their corresponding receptors (CR1, CR3, and CR4) present in several cell types like macrophages, neutrophils, etc. B lymphocytes also express complement receptors, like CD35 (CR1) and CD21 (CR2) [60]. CR1 binds C3b and C4b, while CR2 binds C3d. It is known that CR2 interacts with CD19; this complex acts as a co-receptor for BCR [61].

Complement receptors CR1 and CR2 are expressed differentially during B-human-lymphocyte development. CR1 is more expressed in memory cells than in naïve cells, while CR2 is low expressed in memory cells. Changes in CR expression on B lymphocytes are related with breaking of B-cell tolerance and increased susceptibility to bacterial infections [62]. CR participates in bacteria internalization; *Francisella tularensis* is engulfed by B lymphocytes through complement receptors [63].

3.5. Fc receptors

Fc receptors (FcRs) belong to the immunoglobulin superfamily; they are expressed in many immune cells as monocytes, mastocytes, NK cells, and B lymphocytes and recognize the cristalizable fragment of the immunoglobulins (Fc) [64]. FcR activation results in many relevant responses like cytotoxicity, phagocytosis, mast cell activation, and pathogen clearance. FcRs are classified according to the antibody isotype; they recognized FcRγ (IgG), FcRε (IgE), and FcRα (IgA) [65].

FcR activation regulates many B lymphocyte activities, like activation and proliferation, class switching, and maturation of naive cells into plasma cells [65, 66]. Alterations in FcR functionality have been related to autoimmune diseases [67]. Some pathogens require immunoglobulin opsonization to be uptaken by B lymphocytes, coxsackievirus—opsonized with non-neutralizing antibodies infects and then replicates into B cells [68]. Bacterial pathogens like *Brucella abortus* require IgM opsonization to be internalized into B lymphocytes [69].

3.6. Mechanisms of bacterial uptake exerted by B lymphocytes

3.6.1. Phagocytosis

Phagocytosis was first described by Mechnikoff in 1884 and as a whole is one of the most important defense mechanisms against pathogens [1]. B lymphocytes have been considered as non-phagocytic cells or with much less internalization capabilities than macrophages [70, 71]. Still, it is a controversy upon the endocytic mechanism that the B lymphocyte exerts to internalize bacteria; it is not yet defined if internalization is by phagocytosis or by macropinocytosis (which is also known also as phagocytosis-like) or other mechanisms [72–74]. Recent evidence has shown that the B1-a lymphocyte subset can internalize pathogens [69, 75]; even some authors readily consider that B1-a lymphocytes are phagocytic [76]. However, still, it is not clear if bacterial recognition by B-lymphocyte receptors like BCR, FcR, CR, dectin-1, etc. activates the B cell to internalize the bacteria or if bacterial uptake is an active process triggered by bacterial components, as is the case of products from the pathogenicity islands of *Salmonella* [77].

In phagocytic cells (macrophages, neutrophils, monocytes), phagocytosis can be divided into type I and type II phagocytosis [78]. Type I phagocytosis is mediated by Fc receptors which recognize targets opsonized by immunoglobulins, being the most important the receptors FcRγ that recognizes different subclasses of IgG bound to the pathogen. Interaction between FcγR and IgG triggers phosphorylation of specific tyrosine residues in the ITAM-type motifs present in the intracellular domain of FcγR [79]. Later, the recruitment and activation of signaling proteins belonging to the Rho GTPases family, like Cdc42, resulting in actin polymerization, membrane protrusion formation, and bacterial internalization occur [80].

In the phagocytosis type II, particles opsonized by several complement fractions (like C3b and iC3b) are recognized by cells that have complement receptors CR1 and CR3 (CD11b), like macrophages or neutrophils. Complement-opsonized particles "sink" into the phagocyte; membrane disturbance is minimal without long membrane protrusion formation; particle internalization does not usually lead to an inflammatory response or oxidative burst. In this case, the small GTPase molecule activated is the GTPase RhoA, and they do not necessarily involve Cdc42 and Rac; after GTPase activation, the remodeling of the membrane driven by filamentous actin, phagosome closure, and pathogen internalization occurs [78, 79, 81].

Up today there are few studies on B-cell capabilities for bacterial internalization; there are descriptions of B-cell internalization of opsonized bacteria with immunoglobulin and with complement [63, 69, 74], but also there are descriptions that B cells can uptake non-opsonized bacteria [72]; so far there are no studies that clarified the role of small GTPases involved in bacterial internalization by B cells that could help to clarify if bacterial uptake by B cells is a phagocytic type I or type II process or if it is a unique endocytic mechanism for B cells.

3.6.2. Clathrin-mediated endocytosis (CME)

Clathrin-mediated endocytosis is a well-known endocytic mechanism performed by many cells, is involved in the intake of extracellular molecules recognized by cell membrane receptors,

and is a major route of traffic from plasma membrane to endosomes [81, 82]. B cells recognize antigens by adaptive and innate mechanisms; the adaptive recognition is the most studied, also in the case for recognition of soluble antigens [83, 84]. Antigen adaptive recognition of B cells involves the antigen-specific B-cell receptor (BCR) expressed by B lymphocytes; BCR has two distinct tasks: the first one is to trigger cell activation after interaction with the specific antigen, and the second one is to internalize the antigen for subsequent processing and presentation on MHC class II molecules [85]. Clathrin is the scaffold of conserved cellular structures (pits) that are formed to capture membranal fractions where various cellular receptors are concentrated; once the ligand is bound to the membrane receptor, clathrin polymerization occurs resulting in a covered pit that detaches from the membrane as a coated vesicle that initiates an endosomal trafficking process [81, 82]. In B lymphocytes, clathrin-mediated endocytosis is a fundamental mechanism to translocate BCR-antigen complex to endosomal compartments; lipid rafts, microfilaments, and dynamin are required for this process [86, 87].

B lymphocytes can recognize bacterial antigens by their BCR, that is the case for *Salmonella* and *Francisella* pathogens. BCR-mediated internalization of *Salmonella Typhimurium* allowed bacterial internalization, antigen presentation into MHC class II molecules, and antibody production against *Salmonella* [88]. For the case of *Francisella*, evidence has demonstrated that *Francisella* is recognized by the B1-a cell by their BCRs alone; meanwhile, in the B1-b- and B2-cell subsets, bacterial recognition required simultaneous participation of BCR and complement receptors CR1 and CR2. *F. tularensis* was internalized by B cells at low rate, and internalized bacteria survive intracellularly [63]. In both studies the internalization process resembled more a cell membrane protrusion formation mechanism (like phagocytosis or macropinocytosis) rather than a clathrin-coated endocytosis mechanism. The size of the clathrin-coated pits is around 120 nm, being too small for a bacteria to fit in; however, recent studies have demonstrated that other larger structures coated with clathrin (clathrin plates) [82] are also formed into the cells; it will be interesting to find if bacteria could be uptaken by this mechanism.

3.6.3. Macropinocytosis

Macropinocytosis is a type of pinocytosis described for Warren Lewis in 1931 [89]. Eukaryotic cells have the capacity to internalize fluid (pinocytosis) and particles (phagocytosis) from the extracellular environment, using a variety of different processes [81]. Micropinocytosis is a common process in all cells; this mechanism results in the formation of small vesicles coated with clathrin, caveolin, or other proteins; also clathrin- and caveolin-independent mechanisms exist [81, 90]. Internalization of larger volume of fluids is mediated by a process called macropinocytosis; many signals trigger macropinocytosis, such as macrophage colony-stimulating factor-1 (CSF-1), epidermal growth factor (EGF), and phorbol myristate acetate [91]. Depending on the cell type, macropinocytosis can be a constitutive or an induced process. Macrophages and dendritic cells often utilize macropinocytosis to screen the extracellular environment for pathogenic or harmful materials [92]. Macropinocytosis is the main route for extracellular fluid uptake by the cells; it depends on energy and actin cytoskeleton rearrangements leading to the formation of filamentous and branched actin, supporting membrane modifications

and lamellipodia formation; actin polymerization is initiated by the activation of the small Rho GTPase family (Rho, Cdc42, and Rac) working in parallel with phosphoinositides, to activate the WASp/Scar proteins, and the Arp2/3 complex, allowing actin branching, that force out plasma membrane and form membrane ruffles [93]. Macropinosomes are formed when these ruffles collapse with the plasma membrane enclosing a large volume of extracellular fluid phase; macropinosomes are spacious vesicles within the cytoplasm that can reach a size >0.2 μm [81]. Some pathogens take advantage of the macropinocytosis mechanism to enter into the non-phagocytic cells; *Shigella*, *Salmonella*, and *Mycobacterium* are bacteria known to use this mechanism [94–97]. The internalization process is characterized by the formation of actin-rich membrane protrusions known also as ruffles; for that, *Salmonella* produces the type III secretion system (T3SS) that translocate effectors from the *Salmonella* pathogenicity island I (SPI) into the host cytosol; these virulence factors target host mediators involved in cytoskeleton rearrangements like Rac, Cdc42, phosphoinositides, Arp2/3 complex, etc., resulting in actin cytoskeleton rearrangements and membrane ruffling [94, 98]. In the process of ruffling formation, the pathogen is captured among the ruffles that finally enclose the bacteria into a spacious macropinosome [93]. B lymphocytes can also be infected by bacterial pathogens that enter the B cell through a mechanism of macropinocytosis; *Salmonella*, *Francisella*, and *Mycobacterium* are some of these pathogens [28, 72, 99]; so far there is not a detailed description of the characteristics of this process in B lymphocytes.

4. Bacterial infections of B lymphocytes

B lymphocytes are central cells of the immune response being responsible of antibody production, but they are also cells that may modulate the immune response by the production of proinflammatory as well as anti-inflammatory cytokines [100, 101]. B lymphocytes can be the target of infection by various pathogens; the most recognized B infections are viral infections, especially infection caused by the Epstein-Barr virus (EBV). The susceptibility of B lymphocytes to this virus is primarily due to the expression of the CD21 molecule that is related to the gp350 viral protein [102]. In B lymphocytes EBV presents the lytic phase [103], while the latent phase of the virus is expressed in memory B lymphocytes [104]. B lymphocytes are also susceptible to viral infections caused by cytomegalovirus [105, 106] and smallpox virus [107, 108], among others. Viral infections in B lymphocytes modify the lymphocyte response to allow viral multiplication or persistence, alter apoptosis processes, interfere with antigenic presentation, etc., all to promote viral survival [55].

Although virus was among the earliest recognized pathogens with infective capabilities toward lymphocytes, perhaps the classic concept that B lymphocytes lacked phagocytic capabilities did not allow the recognition of bacterial infections in these cells [88, 109]. However, at present it is known that lymphocytes can internalize bacterial pathogens (mainly intracellular) and that these infections trigger different B-cell responses. Among the bacterial pathogens recognized with capacity to infect B lymphocytes are the genera: *Salmonella, Brucella, Francisella, Moraxella*, *Mycobacterium*, etc. Some characteristics of such infections will be described.

4.1. *Moraxella* B-cell infection

Moraxella catarrhalis is a Gram-negative bacterium that causes respiratory infections in children and causes chronic lung disease in adults; in children, it is highly associated with ear infections [110, 111]. *M. catarrhalis* produces the superantigen Moraxella IgD (MID)-binding protein, which binds to B-lymphocyte IgD, inducing lymphocyte proliferation, but cell proliferation requires also engagement of the innate TLR receptors [112]. In the particular case of *M. catarrhalis*, TLR-9 is required for B-lymphocyte proliferation; TLR-9 recognizes CpG motifs, distinctive of DNA of bacterial or viral origin [113]. The tonsil B lymphocytes are able to internalize *M. catarrhalis* by receptor-mediated endocytosis and after some time eliminate the internalized bacteria [112]. However, it has been described that *M. catarrhalis* is able to persist in pharyngeal lymphoid tissue including the adenoids and the tonsils, residing in macrophages and B lymphocytes [114].

4.2. *Brucella* B-cell infection

Among facultative intracellular microorganisms, *Brucella* spp. is one of the most representatives; this bacterium causes infections that become persistent in both humans and animals, representing a global zoonosis [115]. *Brucella* persists and replicates primarily in tissues and cells of the mononuclear phagocytic system, such as the spleen, bone marrow, lymph nodes, spleen, macrophages, and dendritic cells, and may also reside in cells of male and female reproductive systems including the uterus, placenta, and ovaries [116]; however, it has recently been recognized that B lymphocytes are not only infected but function as a reservoir of these bacteria [69]. *Brucella* also invades non-phagocytic cells such as epithelial cells, and the mechanism of internalization has been reported as zipper-like, promoting cytoskeleton rearrangement through the activation of Cdc42 by the pathogenic strains of *B. abortus* [117]. *Brucella* internalization into B cells depends on microfilaments and once internalized is allocated into late endosomal/lysosomal compartment allowing bacterial persistence and residency, infected B lymphocyte, thus producing anti-inflammatory cytokines such as TGF-β; interestingly mice lacking of B cell are more resistant to *Brucella* infection, pointing an important role of B cells as immunomodulators toward brucellosis [69].

4.3. *Francisella* B-cell infection

Francisella (especially *F. tularensis*) is another bacterium able to internalize and invade B lymphocytes; this bacterium is a facultative intracellular pathogen and has been reported that it infects phagocytic cells like macrophages, neutrophils [118, 119], and pulmonary and hepatic non-phagocytic cells [120, 121], among others. The main host cells of *F. tularensis* are the macrophages, where once the bacterium is internalized, it inhibits phagolysosomal fusion and leaves the cytoplasm, where it resides and actively replicates [122–124]. *Francisella*-infected macrophages promote a systemic anti-inflammatory reaction with high levels of TGF-β [125], with fatal consequences for the infected individual [126]. The first studies that highlighted the involvement of B lymphocytes in *F. tularensis* infection date back to the late 1990s, where it was found that B cells rather than antibodies were critical for protection against this bacterium [127].

The infective abilities of *F. tularensis* toward B lymphocytes were described in 2008, and it was found that the bacterium could be readily internalized by primary B lymphocytes and by B lymphocyte cell lines like Ramos or A20; lymphocytes allowed a moderate intracellular multiplication by 24 h after infection; however, from this time infected lymphocytes began to undergo apoptosis, which was accentuated at 48 h postinfection [128].

Among the B-lymphocyte (CD19+) subtypes, B1-a lymphocytes, classified as innate lymphocytes, are the best infected by *F. tularensis*, requiring only the BCR engagement for bacterial internalization, whereas B1-b and B2 lymphocytes are also susceptible to infection, although to a lesser extent; they require the joint participation of BCR and CR1/CR2 receptors for bacterial internalization [63]. B lymphocytes from mice infected with the live vaccine strain of *F. tularensis* (LVS), especially the B1-a lymphocytes uptake the bacteria; produce numerous proinflammatory cytokines such as IFN-γ, IL-1β, IL-12, IL-17, and TNF-α; decrease amounts of IL-10; and express costimulatory molecules like CD80 and CD86; mice were able to clear bacterial burden 10 days postinfection [129]. Recent studies confirm that the involvement of B lymphocytes is critical for the control of *F. novicida* infections [130] and that *Francisella* internalization into B cells requires cell membrane integrity [63].

4.4. *Salmonella* B-cell infection

The role of B lymphocytes among the bacterial infections has been studied the most for the case of *Salmonella* infection, and the evidences demonstrate the importance of these cells in the pathogenesis of diseases caused by this bacterial genus. Infections caused by *Salmonella* genus can be differentiated into two types: typhoid and non-typhoid. Gastroenteritis are caused by non-typhoidal serovars such as *Salmonella enterica* serovar Typhimurium, *S. enterica* serovar Enteritidis, and *S. enterica* serovar Newport, while the enteric fever is caused by typhoidal *Salmonella* serovars like *Salmonella enteritidis* serovar Typhi and *S. enteritidis* serovar Paratyphi [131]. The infection caused by *S. Typhimurium* in mouse is very similar to that caused by *Salmonella* Typhi in the human, and much of the knowledge of this infection is due to studies in the murine model with *S. Typhimurium* [132]. *Salmonella* are facultative intracellular pathogens; both serovars share many virulence factors (flagella, lipopolysaccharide, and pathogenicity islands) but differ in clinical manifestations: typhoid fever occurs within 2 weeks and has systemic manifestations, and gastroenteritis occurs in a shorter period (12–72 h), with a rapid accumulation of neutrophils at the intestinal level [133]. After *S. enterica* enters the organism orally, it is rapidly captured by the epithelial cells of the intestine and M cells and after a few hours is found in the lamina propria of the intestine and in the Peyer's plaques [134]. Already in the intestinal tissue, Salmonella is internalized by various phagocytic cell types such as macrophages, dendritic cells, and neutrophils [135], and it has been suggested that Salmonella is internalized also by B lymphocytes, which are abundant in Peyer's plaques adjacent to intestinal M cells [43, 136]. *Salmonella* epithelial cell invasion is an active process triggered by the bacterium, in which cell cytoskeleton rearrangements OCCUR, resulting in the formation of membranal protrusions that allow bacterial internalization, a phenomenon known as macropinocytosis [137]; *Salmonella* has developed very specialized systems to promote this event, within these is the type III secretion system (TTSS), through which the bacteria injects to the host cell products

derived from the pathogenicity islands I and 2 (SPI1, SPI2) [138], which promote the activation of Rho, Rac, and Cdc42 small GTPases, thus favoring cytoskeletal remodeling and stimulating the production of caspase-1 which catalytically activates proinflammatory cytokines such as IL-1β and IL -18 [133]. Once internalized, *Salmonella* resides intracellularly in membranes called *Salmonella*-containing vacuole (SCV), which protects the bacteria by avoiding fusion with lysosomes and avoiding the reactivity of reactive oxygen metabolites [139, 140]. *Salmonella* can infect several cell types, from macrophage and dendritic cells to non-phagocytic like epithelial cells and hepatocytes [141]. *Salmonella* can be internalized in and infect B lymphocytes [43]. Evidence has shown that *Salmonella* is internalized into B lymphocytes through a macropinocytosis process [72, 73]. Bacterial recognition by B cells is also required for internalization; *Salmonella* may be recognized by BCR [88], by TLR [142], or by products derived from SPI-1 [143]. Depending on the internalization mechanism, *Salmonella* survival will occur, for instance, bacteria opsonized with complement or internalized by a mechanism triggered by products of the SPI-1 survive a replicate few hours after internalization, whereas bacteria opsonized with IgG or not opsonized will be eliminated soon after internalization [139]. Once internalized, the bacterium resides in SCV vacuoles, which in the case of B lymphocytes allow the cross-presentation of the Salmonella antigens to major histocompatibility complex molecules class one (MHC-I), by the vacuolar or cytosolic pathways [144]. This cross-presentation would promote infected-B-cell recognition and elimination by CD8 + T lymphocytes; however, this elimination does not occur, and it has been described that in both B1 and B2 lymphocytes infected with *Salmonella*, the PD1-PD1L pathway (programmed death-1; programmed death-1 ligand) is expressed, resulting in a reduction in the signaling required for activation of the T-cell receptor (TCR) and consequently avoiding B-cell death [145–147]. One of the characteristics of *Salmonella* is its ability to persist, and the use of the PD1 system in B lymphocytes makes them an ideal niche for prolonged stay in the body.

Thus, B lymphocytes play a key role in *Salmonella* infections, functioning as bacteria reservoir, acting as immunoregulatory cell through IL-10 production [148], facilitating bacterial systemic dissemination [43], and promoting a proinflammatory intestinal state characteristic of non-typhoidal infections through the production of proinflammatory cytokines such as IL-1β [135].

4.5. *Mycobacterium* B-cell infection

The genus *Mycobacterium* comprises a large number of species, some of which are highly pathogenic as *Mycobacterium tuberculosis* (MTB), being also the most studied species of all mycobacteria. MTB is a facultative intracellular pathogen; macrophages are the main cells where the mycobacteria reside and multiply [149], being able to infect other cells such as pulmonary epithelial cells [93, 150], fibroblasts [151], adipocytes [152], or endothelial cells [153]; bacterial replication into the non-phagocytic cells is discrete, so they have been suggested as niches where the bacteria may persist. In mycobacterial infections, the protective response is cellular, mediated by T helper (Th) lymphocytes and activated macrophages [154]. The involvement of antibodies and B lymphocytes has recently begun to be recognized. For example, B lymphocytes

are required to control pulmonary inflammation and bacterial load [155] and antibodies and cytokine production by B lymphocytes mainly IL-10 and contribute to these activities [156]. Lymphocytes have been considered as non-phagocytic cells or with less interiorization capacity than macrophages [70, 71]. *Mycobacteria* promote their internalization in non-phagocytic cells, including B lymphocytes [72]. One way to establish the low phagocytic activity of B lymphocytes is to incubate them in the presence of inert particles like zymosan (**Figure 4**).

Macropinocytosis is an internalization process triggered by several inductors [157]; experimentally, phorbol esters trigger macropinocytosis even in phagocytic cells [158]. Pathogens use this internalization mechanism to achieve their entry into cells, by producing factors that trigger cytoskeleton reorganization [93]; for the case of mycobacteria, our group has suggested that pathogenic mycobacteria such as *M. tuberculosis* or nonpathogenic *Mycobacterium smegmatis* produce soluble factors present in the culture medium that trigger this phenomenon in B lymphocytes (**Figure 5**). Some of the reported mycobacterial products that facilitate adhesion and internalization into non-phagocytic cells are fibronectin-binding protein (FBP) and heparin-binding hemagglutinin adhesin (HBHA) [159, 160], among others [161, 162]. The internalization of mycobacteria in immortalized B lymphocytes (cell lines) has been described by some authors [72, 74, 163]; these studies show that *M. tuberculosis* survives and multiplies intracellularly in B lymphocytes, and as a consequence of infection, there is lymphocyte activation, resulting in antibody production of IgM class mainly and expression of co-stimulatory molecules like CD80 and CD86 [74]. There are scarce studies on human in vivo B-lymphocyte infection [164], so establishing the precise involvement of B lymphocytes in mycobacterial infections is an area of great interest.

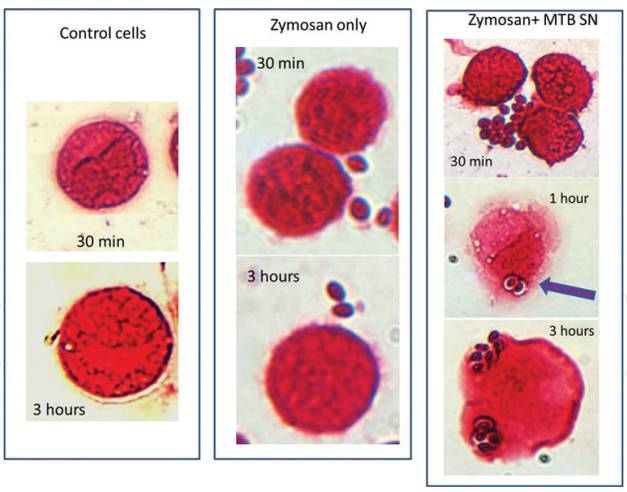

Figure 4. Is the B cell a "phagocytic cell"? B cells from the Raji cell line were incubated with zymosan without any further treatment, during 3 h; then cells were fixed with paraformaldehyde and stained with Giemsa dye. Control cells: cells did not receive any treatment. Zymosan only: cells were incubated with zymosan without any treatment. Zymosan and MTB SN: cells were incubated with zymosan and *Mycobacterium tuberculosis* filtrated growth bacterial culture medium (0.22 mm) for complete removal of the bacteria. Zymosan only: zymosan particles were observed bound to B-cell membrane; 3 h after, zymosan was not observed into the B cells. Zymosan + MTB SN: culture medium collected after 2 weeks of incubation at 37°C was placed with B lymphocyte; more zymosan particles were observed bound to B cell; after one h it was possible to observe intracellular zymosan.

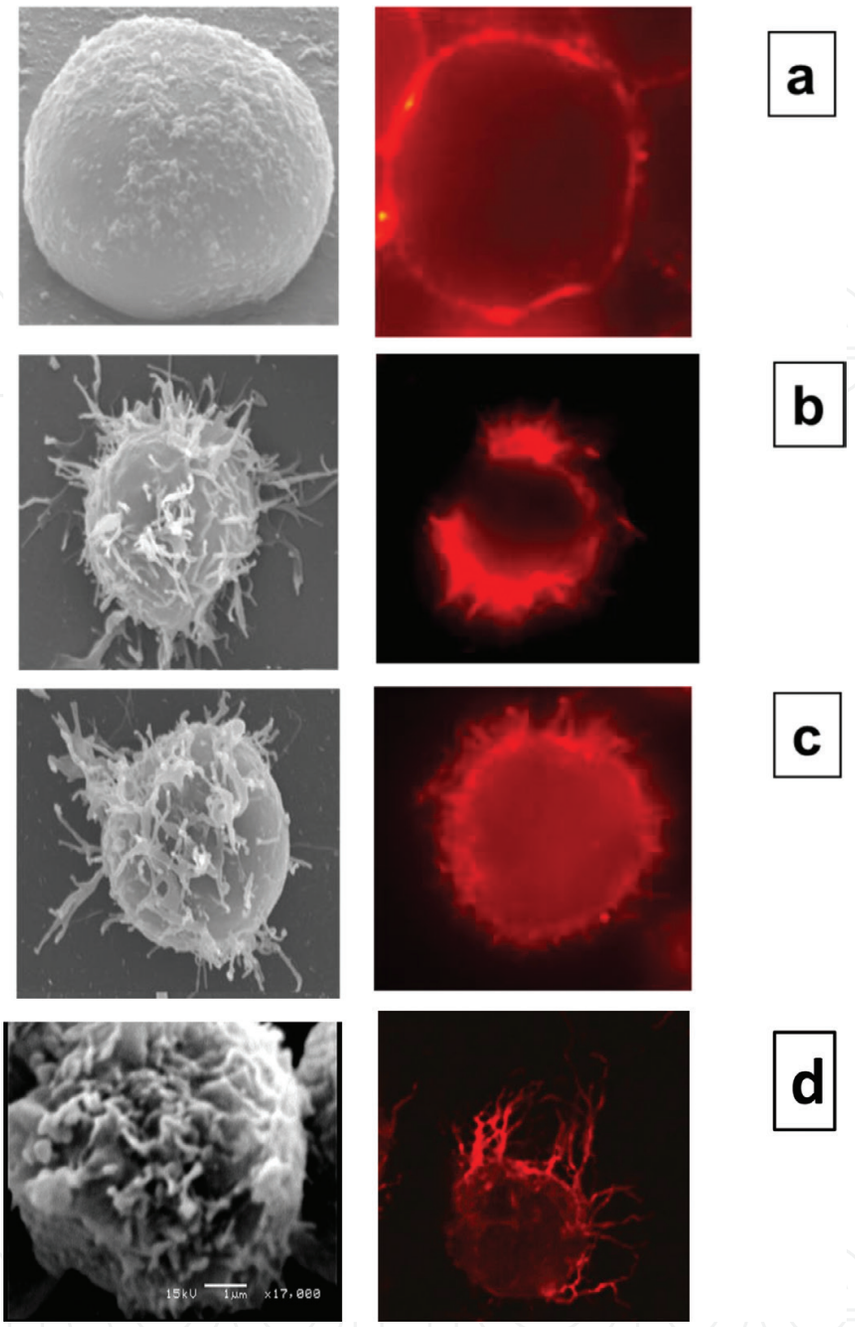

Figure 5. B-cell membrane changes after 1 h of incubation with PMA or mycobacteria derivatives. Scanning electron microscopy (SEM) and fluorescence microscopy images of Raji B cells. (Panel a) Control cells. (Panel b) Cells treated with phorbol-myristate-acetate (PMA), a classical macropinocytosis inductor. (Panel c) Cells treated with filtrated supernatant from growth culture medium of *Mycobacterium smegmatis*. (Panel d) Cells infected with *Mycobacterium tuberculosis*. Fluorescence images correspond to actin cytoskeleton labeled with phalloidin rhodamine. SEM images were 17,000× (panels a and d) or 15,000× (panels b and c); all fluorescence images were observed at 1200×.

5. Implications of B-cell response in bacterial infections

B lymphocytes are involved in various stages of the immune response against pathogens; the traditional role of these cells has been associated with adaptive immune response, characterized

by the production of antibodies and the generation of immune memory. In bacterial infection, the role of these cells in the innate response has recently been recognized; in this sense, it has been demonstrated that B cells express receptors capable to recognize bacterial structures (TLRs, CR, dectin-1, etc.) [55, 165–167] and produce the effectors of the immune response—cytokines and antimicrobial peptides [168–171]—also activating mechanisms that prompt pathogen control like nitric oxide (NO) and antimicrobial peptides, among other [168]. The immune response induced in B lymphocytes often depends on the type of pathogen and the way in which B cell is activated, so B-cell response may be regulated by the bacteria to favor its intracellular survival. The B lymphocytes possess an endocytic capability that allows them to internalize pathogens; the mechanisms that these cells use to internalize bacteria can be endocytosis dependent or independent of clathrin, macropinocytosis, phagocytosis, etc. [72, 73, 88, 88, 168, 172]. As a result of this internalization, B lymphocytes produce a series of mediators of the innate response that will be described.

5.1. Cytokines

B cells recognize pathogens in an infectious process through the BCR or PRR receptors; this recognition may induce the cell activation and the production of inflammatory cytokines such as IL-1, IL-6, IL-8, TNFα, and IFNγ and suppressor cytokines as IL-10 and transforming growth factor beta (TGF-β), in addition to participating in Th2 profile events characterized by the production of IL-4, IL-5, IL-13, granulocyte-colony stimulating factor (GCSF), and granulocyte-macrophage colony-stimulating factor (GMCSF). B-cell plasticity is so extensive that any of these profiles can be induced by B cells depending on how they are activated [173–176].

Although B cells can show an inflammatory profile in response to bacterial infections, they cannot control infection at all times; some pathogens, especially intracellular ones, are able to occupy B cells as reservoirs of infection and can modulate the immune response of these cells to survive or even multiply within B lymphocytes. In the case of B-lymphocyte interaction with some Gram-negative bacteria such as *Brucella abortus* [69], *M. catarrhalis* [112, 166], and *Salmonella spp* [148, 177, 178], induce a high production of IL-10 which activates an immunosuppressive response characterized in addition by the simultaneous production of TGF-β, under this stage the bacteria rapidly spread. *Listeria monocytogenes* infection shows an IL-10-producing B-cell profile at very early stages of infection, which promotes bacteria persistence and dissemination [179–181]. Another example of B lymphocyte-bacteria interaction is the infection caused by *F. tularensis* which in B cell (particularly B1-a subtype) induces a clearly inflammatory profile characterized by the production of IL1-b, IFNγ, IL-6, IL-12, IL-17, and TNF-α [128, 129]. Regarding the activation of the inflammasome complex in B cells infected with *Salmonella*, it has been described that although this system is functional in B cells, the IL1-β is not secreted because this bacterium inhibits its production by a mechanism of negative regulation of the NLRC4 protein, which favors bacterial intracellular persistence [182].

5.2. Nitric oxide (NO)

Nitric oxide is one of the important mediators of the immune response that plays a fundamental role in the elimination of pathogens. This molecule is produced by classical phagocytic

cells; however, its production has also been described by cells classified as non-phagocytic including B lymphocytes. During respiratory burst, NO in conjunction with the reactive oxygen species (ROS) participates in the formation of peroxynitrites, which are highly oxidizing agents of many components of the bacteria. NO increases its expression and activity in B lymphocytes infected with intracellular pathogens such as *M. tuberculosis*, *S. Typhimurium*, and *Citrobacter rodentium* [183–185]. The subclass of B1 lymphocytes constitutively produces nitric oxide inducible synthase (iNOS); however, in infectious events this enzyme increases its expression levels and therefore its activity, such as the LBs infected with *Cryptococcus neoformans*; in this infection, NO has a fundamental role in the elimination of the pathogen [186]. NO production in B1 lymphocytes appears to be linked to the stimulation of various TLRs, since some studies have shown that the stimulation of these receptors and their ligands resulted in production of higher NO levels by B lymphocytes. Of the Toll receptor ligands that have been studied, the major enhancer of NO expression was bacterial LPS; other agonists such as Poly I: C (TLR3), Imiquimod (TLR4), and CpG DNA (TLR9) also induce their expression [168, 187].

5.3. Antimicrobial peptides

Antimicrobial peptides are innate response effectors present in most human cells; these molecules are classified into alpha-defensins (HNP1-6) beta-defensins (hBD 1-4), and cathelicidins such as LL37. Its mechanisms of action include the direct lysis of the microorganisms, the generation of a proinflammatory environment, or the modulation of the immune response. There are very few studies on B-lymphocyte expression of antimicrobial peptides; however, there are some evidences demonstrating that B cells express antimicrobial peptides in constitutive and inducible fashion; under stimulation with some PAMPS, B lymphocytes express alpha defensins (HNPs 1–3), hBD2, and the cathelicidin LL-37 [170, 188].

5.4. Reactive oxygen species

B cells participate actively in the control of microorganisms, and although many authors have considered them as non-phagocytic cells, it seems that these cells possess microbicidal capacities, since they are able to produce antibacterial mediators like ROS. The Nox family of enzymes is responsible for regulating the production of ROS in several cell types like neutrophils and macrophages; the Nox2 isoform is particularly essential in the elimination of bacteria in these cells. Recently Nox2 production was described by splenic and peritoneal B lymphocytes; the absence in Nox2 production decreases the production of ROS resulting in a deficient elimination of *Staphylococcus aureus* by B lymphocytes; contrarily normal B cell controlled intracellular bacteria growth [171].

5.5. Regulation of B-cell survival during bacterial infection

The ability of several pathogens to regulate the death pathways of the host cell has been described for most of the pathogens that infect different cells, and actually this situation is recognized for B lymphocytes. For example, *L. monocytogenes* is known to induce apoptosis in B cells through various mechanisms such as activation of caspases 3, 8, and 9 [179, 189]. Apoptosis

induction during bacterial infection of B cells has been reported for *F. tularensis*, *S. flexneri*, and *Helicobacter pylori* [55, 128, 190]; in all three cases, apoptosis of B cells is a mechanism that can facilitate the survival and dissemination of pathogens in their host due to the death of B lymphocytes that could have a protective effect. In comparison, during B-cell infection with *Salmonella*, the bacteria promote B-cell survival by engaging different mediators like PD-1 [145] or through the negative regulation of the protein NLRC4 [182], avoiding in both cases cell death. In this way, B cell becomes an intracellular niche for bacterial survival, persistence, and dissemination; the bacteria have a major role in promoting this situation [43, 182].

6. Concluding remarks

B lymphocytes are fascinating cells, far beyond being precursors of plasma cells; they represent a heterogenic cell population with an ample range of activities; beside antibody synthesis, they act as antigen-presenting cells; they produce pro- and anti-inflammatory cytokines acting as modulators of the immune response; and also they can uptake bacterial pathogens for latter processing and presenting them to T cells. Recent findings have left behind the old idea that B lymphocytes were not able to internalize bacterial or particulate antigens, but not all the B lymphocyte subsets have this ability. The B1-a subset is the major B-cell subset that is able to internalize bacterial pathogens; in the beginning, the antigen is recognized by PRRs like BCRs, TLRs, CRs, etc. After recognition, B cell will be activated, internalizing the bacteria, and depending on antigen´s nature (pathogenic or no pathogenic), the B lymphocyte will be activated to contribute in the establishment of a protective immune response. In some cases (specially for pathogenic bacteria), the B cell will not be able to control the pathogen; then the B cell becomes a pathogen niche or reservoir, acting as a "Trojan horse" that allows bacteria dissemination in the organism; in other cases, the pathogen modulates B-cell death, allowing cell and pathogen survival and making the B cell as an excellent host for bacterial persistence. The endocytic pathways performed by B lymphocytes to uptake bacteria, so far reported, are macropinocytosis and phagocytosis; still, there exists controversy regarding the ability of B lymphocyte to perform one, the other, or both mechanisms. It has been proposed that the B1-a cell subset has the double lineage lymphocyte/macrophage, making the B1-a cell prompt to respond to bacterial challenges and to respond to them in conjunction with the T cells; then these cells represent a bridge between the innate and the adaptive responses. Still, the B cells have many "secrets" that have to be revealed.

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