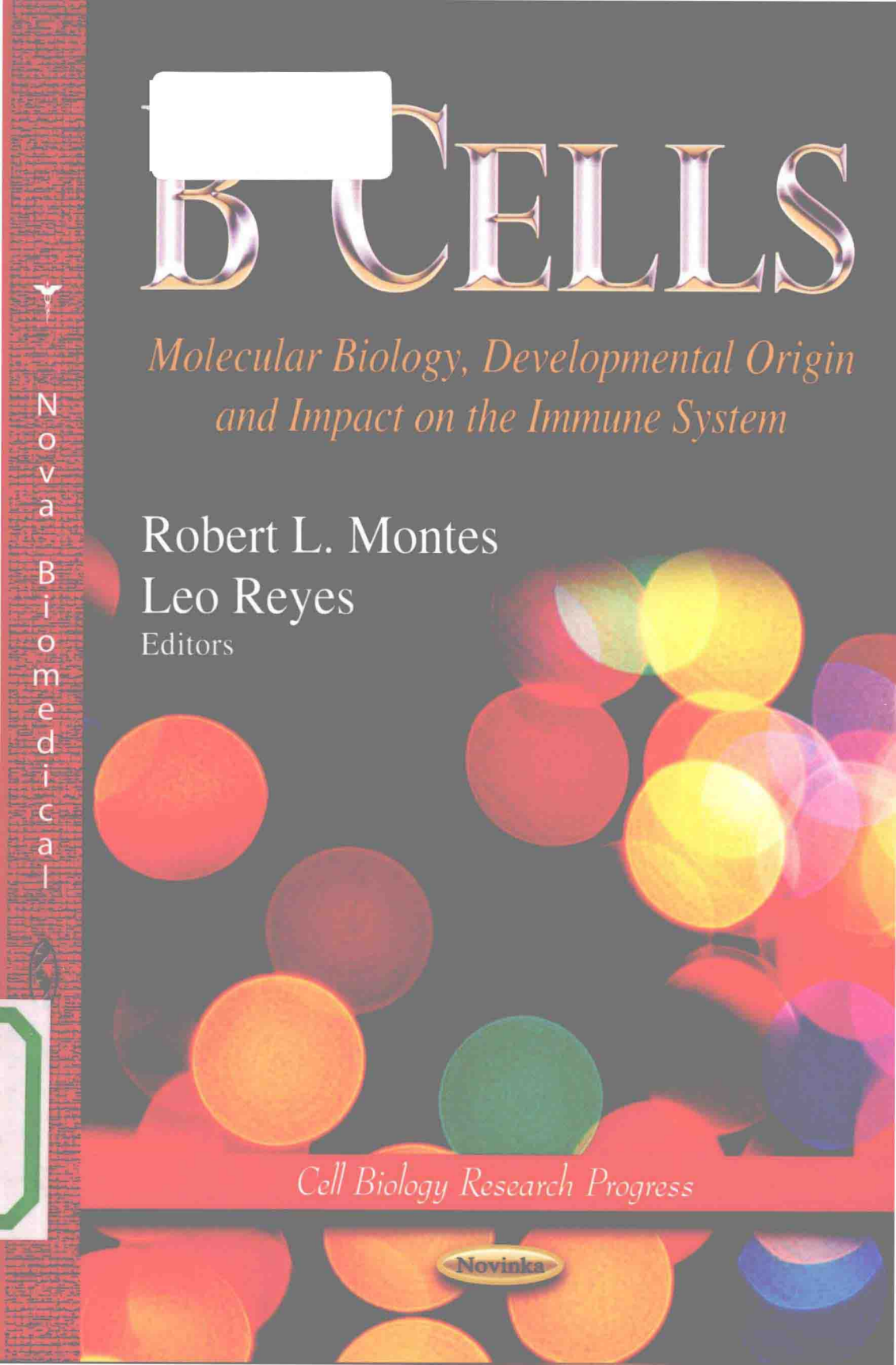
B CELLS

Molecular Biology, Developmental Origin and Impact on the Immune System

Robert L. Montes
Leo Reyes
Editors



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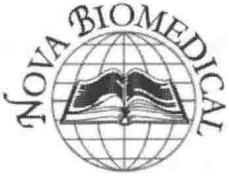

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Preface

B cells belong to a group of white blood cells known as lymphocytes, making them a vital part of the immune system. The human body makes millions of different types of B cells each day that circulate in the blood and lymphatic system performing the role of immune surveillance. They do not produce antibodies until they become fully activated. In this book, the authors present current research in the study of the molecular biology, developmental origin and impact on the immune system of B cells. Topics discussed include the role of B cells in intracellular bacterial pathogen infections; dynamic clustering of BCR-lipid rafts in antigen affinity discrimination by B cells; and B cells as a key player in the pathogenesis of multiple sclerosis.

Chapter I – The protective immune response against facultative intracellular bacterial pathogens, as are *Brucella melitensis*, *Francisella tularensis*, *Listeria monocytogenes*, or *Salmonella sp.* requires concerted action of activated immunocompetent cells. In this respect the dominant role has been attributed to activated T lymphocyte subpopulations and/or activated macrophages. Recently become clear that the B cell subsets are the equivalent partner of T cells during the induction, regulation and expression phases of the immune response against intracellular bacteria. They express their functions by both antibody-dependent and by antibody-independent mechanisms. B cells are able to internalize bacteria through their BCR, extract the antigens from the non-internalizable particles, and are able due to the cross-presentation process activate $CD4^+$ as well as $CD8^+$ T cells. Through the dichotomy of the recognition of bacterial targets by BCR or TLR and by the production of sets of cytokines the B cells regulate the expression of individual branches of acquired immune response. B cells also influence the development of T cell memory. Further, B cells apply their dominant role as the antibody producing

cells for influencing the course and final resolution of intracellular bacterial infections. Direct interaction of B cell subpopulations with bacteria results in autonomous B cell differentiation and rapid secretion of bacterium-specific antibodies that influence the pathogen distribution to vital organs. Moreover specific antibodies against intracellular bacteria have been demonstrated to have protective potential. On the other hand the intracellular bacteria survive intracellularly in B cells mainly in non-replicative state and as such constitute the source of systemic infection and/or reservoir for reinfection. B cells thus represent one of the cell types that are responsible for the final fate of the interaction of intracellular bacterial pathogens with their hosts. In this review the authors summarize the studies on several infectious models documented the beneficial as well as relatively deleterious role of B cells in the course of intracellular bacterial infections.

Chapter II – Lymphocytes (B and T cells) have the ability to mount an appropriate immune response based on their immune receptor's affinity for antigens, a feature known as affinity discrimination. In B lymphocytes, affinity discrimination is characterized by monotonically increasing signaling response with increasing affinity. Thus selection of higher affinity antibodies through the process of affinity maturation leads to heightened humoral response and imparts an adaptive advantage. The authors have recently utilized a Monte Carlo based computational model to study antigen ligation recognition induced formation of BCR-lipid rafts. Controlled *in silico* experiments indicated that the formation of BCR-lipid rafts, upon antigen ligation, is affinity dependent and generates a mechanism for affinity dependent signaling. In this article, the authors discuss the ramifications of our study of BCR-lipid raft formation on understanding B cell affinity discrimination and various related aspects of B cell activation. The authors also analyze recent experimental findings on affinity discrimination in B cells based on our study of lipid raft formation. In addition, this study allows us consider kinetic proofreading and serial triggering mechanisms in the context of BCR-lipid raft formation, providing further mechanistic insights into the problem of B cell affinity discrimination. The authors discuss the broader implications of our results such as for the affinity maturation process and immunotherapeutic strategies.

Chapter III – B cells have been implicated in the pathology of multiple sclerosis (MS) since the initial observation of increased immunoglobulin concentrations in the cerebrospinal fluid (CSF) of MS patients in the 1940s. CSF oligoclonal IgG bands are detectable in most MS patients, and data on the single cell cloning of the immunoglobulin gene from B cells obtained from MS lesions suggest antigen-driven clonal expansion of B cells within the

central nervous system (CNS). Until recently, the main pathogenic contribution of B cells to MS has been considered to be mediated by the production of autoantibodies specific to CNS antigens. However, the recent successful results of B-cell depletion therapies for MS without affecting immunoglobulin levels in serum and CSF have shed light on the antibody-independent activity of B cells in MS immune pathology. Indeed, studies using animal models and human samples have suggested that B cells mediate peripheral immune regulation of MS by presenting antigens to T cells and by releasing anti- and/or pro-inflammatory cytokines. In addition, the recent identification of B cell-rich follicle-like structures in meninges of patients with secondary progressive MS suggests that the pathogenic roles of B cells also exist within the CNS and that they contribute to subpial cortical injury, which is currently considered an important substrate for disease progression. Taken together, B cells can be regarded as a key player in the pathogenesis of MS and can consequently serve as an attractive target for therapeutic intervention. In this chapter, the authors review the pathogenic roles of B cells in MS, with a focus on recent advances in the field and the potential for future B cell-targeted therapies for MS.

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Chapter I

The Role of B Cells in Intracellular Bacterial Pathogen Infections

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Abstract

The protective immune response against facultative intracellular bacterial pathogens, as are *Brucella melitensis*, *Francisella tularensis*, *Listeria monocytogenes*, or *Salmonella sp.* requires concerted action of activated immunocompetent cells. In this respect the dominant role has been attributed to activated T lymphocyte subpopulations and/or activated macrophages. Recently become clear that the B cell subsets are the equivalent partner of T cells during the induction, regulation and expression phases of the immune response against intracellular bacteria. They express their functions by both antibody-dependent and by antibody-independent mechanisms. B cells are able to internalize bacteria through their BCR, extract the antigens from the non-internalizable particles, and are able due to the cross-presentation process activate $CD4^+$

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as well as $CD8^+$ T cells. Through the dichotomy of the recognition of bacterial targets by BCR or TLR and by the production of sets of cytokines the B cells regulate the expression of individual branches of acquired immune response. B cells also influence the development of T cell memory. Further, B cells apply their dominant role as the antibody producing cells for influencing the course and final resolution of intracellular bacterial infections. Direct interaction of B cell subpopulations with bacteria results in autonomous B cell differentiation and rapid secretion of bacterium-specific antibodies that influence the pathogen distribution to vital organs. Moreover specific antibodies against intracellular bacteria have been demonstrated to have protective potential. On the other hand the intracellular bacteria survive intracellularly in B cells mainly in non-replicative state and as such constitute the source of systemic infection and/or reservoir for reinfection. B cells thus represent one of the cell types that are responsible for the final fate of the interaction of intracellular bacterial pathogens with their hosts. In this review we summarize the studies on several infectious models documented the beneficial as well as relatively deleterious role of B cells in the course of intracellular bacterial infections.

Introduction

One of the fundamental properties of living systems is that they colonize all spaces available to them. This probably is the reason some bacteria colonize spaces within other living systems. Doing so essentially provides entry to a new living space with new sources of nutrients. Therefore, if the surfaces of a host – be they of organs or cells – are for any reason not suitable for survival and proliferation, some pathogenic bacteria enter into the host cell. In terms of lifestyle, it is thus possible to classify pathogenic bacteria into three groups: Extracellular bacteria proliferate on the mucous membranes or cell surfaces, or they have no contact whatsoever with eukaryotic cells. Facultative intracellular bacteria proliferate both outside and inside the host cells. Finally, strict or obligate intracellular pathogens are able to multiply exclusively within the host cells. This group of bacteria in particular poses both serious health problems and scientific challenges. Prophylaxis against some illnesses caused by intracellular bacteria is problematic because the existing vaccines can be used only for livestock and are not currently licensed for human use, as is the case, for example, of those for tularemia or

brucellosis. The bacteria themselves present scientific challenges for the fields of infection biology, molecular microbiology, and immunology.

Obligate and facultative intracellular bacteria have the ability to adhere to cell surfaces, than actively induce the process of internalization, and finally survive and proliferate inside the characteristic intracellular niche of professional phagocytes. It should be noted that there also are bacteria that proliferate inside non-professional phagocytes, but such bacteria cannot be classified according to older criteria as intracellular pathogens. Examples include *Escherichia coli*, with its ability to enter into epithelial cells via invasin AfaD [1]; *Staphylococcus aureus*, which penetrates the cells of mucosa; and *Streptococcus pyogenes* entering the epithelial cells }with both of the latter utilizing fibronectin binding proteins as the tool for cell invasion) [2, 3]. The entry into non-phagocyting cells is vastly more widespread than we have recognized in the past.

The protective response against intracellular bacterial pathogens has long been attributed to activation and expression of the Th1 type of acquired immune response based on the production of IFN-gamma and TNF-alpha and on activation of the mononuclear phagocytic cells that have been considered the basic effector cells needed for resolving infection. Recent research focusing on the function of B cells in the course of intracellular bacterial infections clarly demonstrates that B cells are one of the cell types needed for the expression of an effective protective response. Beyond their historically well-recognized role in mediating humoral immune responses, B cells respond directly to such bacterial surface components as peptidoglycans, capsular polysaccharides, outer membrane proteins or lipopolysaccharides and by a T-independent manner influencing the induction of immune response. Moreover, the peripheral B cells, despite the classical conception of B cells as non-phagocytizing, have been shown to phagocytize inert particles or bacteria when these corpuscles are recognized by the B cell receptor (BCR). Thus, in fact, B cells can be characterized as ligand-selective phagocytic cells [4]. The internalization of antigenic particles, along with the ability to extract the antigens from non-internalizable surfaces [5], is a prerequisite for the function of B cells as antigen-presenting cells, which is also a well-recognized function of B cells within the immune system. At minimum, B1 and marginal zone B cells participate in innate immune responses and may represent the functional link between innate and actively acquired immune responses. Therefore, B cells, in their diversity and complexity, are integral of both natural as well as acquired immune system responses to infectious agents and can also contribute effectively to the phase of immunological memory.

The interaction of B cells with microbes, including intracellular bacteria, have multiple consequences for the B cells themselves and for the induced immune response as a whole. It is well documented that some microbes can directly induce polyclonal B cell activation, leading on the one hand to the proliferation of multiple B cell clones and up-regulation of activation markers, including of MHC class II, and on the other hand to the production of antibodies recognizing heterologous and also autologous antigens [6]. The best characterized tool of B cells for regulating immune responses is the production of IL-10 and TGF-β [7, 8] by the subset of B cells recently termed Breg. This B cell subset expressing CD5 has been shown to be the producer of IL-10 in response to LPS [9, 10]. B cells, similarly to T cells during the induction of acquired immunity, can be subdivided into at least two functional effector subsets producing distinct arrays of cytokines and chemokines. So-called B effector 1 cells (Be-1 cells) produce IFN-γ, TNF-α, IL-12/p40 and IL-10 while B effector 2 cells (Be-2) produce cytokines IL-2, IL-4, IL-6, IL-10, IL-13, and TNF-α. The original resolution to Be-1 or Be-2 cells is dependent on the cognate interaction with Th1 and Th2 cells, respectively. Interestingly, these subsets of B cells can interact with naïve $CD4^+$ T cells and prime them into the Th1 or Th2 immunoregulatory branch of the acquired immune response. Thus, the helper function of T cells and B cells during adaptive immune response is reciprocal and suggest the existence of an amplification loop during establishment of either humoral or cellular types of immune responses [11–14]. Finally, subsets of B cells, recently termed Bmem, participate in the stage of immunological memory that is functional following re-encounter with the cognate antigen [15–19]. Together with long-lived plasma cells, they enhance the host resistance for a long time [20, 21].

Thus, B cells in general can have the potential to be an equal partner for T cells, macrophages, and dendritic cells in the creation of effective protection against intracellular bacteria. This chapter summarizes the recent advances in our understanding of the mutual interaction between B cells and intracellular bacteria and the consequent implications for the induction, regulation, and expression of immune response against this type of infection.

B Lymphocytes Provide a Living Niche for Intracellular Bacteria

The general paradigm that it is phagocytes from among the cells of the immune system that provide the living niche for intracellular bacterial parasites is slowly changing. A specific endocytic compartment differing from all other cellular organelles has been described as the natural living niche inside macrophages for bacteria of the genera *Salmonella*, *Brucella* or *Francisella*, and it either has been given names reminiscent of real organelles [22, 23] or simple names such as "*Francisella*-containing vacuole (FCV)" [24]. However, intracellular bacteria are able also to interact directly with lymphocytes, activate them, and even to enter them. Both human and mice B cells have been shown to bind different species of bacteria, among these including such intracellular bacteria as *Brucella* [25, 26], *Mycobacterium tuberculosis* [27], or *Chlamydia trachomatis* [28], and to activate them to produce polyclonal immunoglobulins and/or cytokines [29, 30]. As early as the 1970s, surface molecules on leukocytes as integrins, lectin receptors [26], MHC-antigens [31], or surface immunoglobulins [32] were identified to bind bacterial ligands. Despite these early studies it took several decades before the attention was turned again to the direct interaction of intracellular bacteria with B cells. A significant reason for the study of B cells has been the accumulating data documenting the significant effects of B cells and/or circulating antibodies on the course of immune response against intracellular bacteria that, until that time, had been underestimated. It should be noted that in this respect we still have only limited knowledge from several bacterial experimental models toward clearly defining the "desirable" and/or "undesirable" effects of B cells in the course of infections caused by intracellular bacterial pathogens.

Brucellosis has been known since antiquity as a chronic, protracted infection in animals and humans. The causative agent of this zoonosis is *Brucella spp.*, a Gram-negative coccobacillus that is a facultative intracellular pathogen. It is generally established that this pathogen resides inside the macrophages and is localized in phago(lyso)somes [33], a pathogen-specific niche that in the literature gained the peculiar designation "brucellosome" [22]. One of the *Brucella* subtypes, *Brucella abortus*, recently has been shown to infect also B cells. Bacteria opsonized by immunoglobulin M and complement survive inside primary murine B cells. The internalization of *Brucella* into B cells is promoted by functional microfilaments. Once inside

the B cell, the *Brucella* resides in a late-endosomal/lysosomal compartment [34]. The association of *Brucellae* with B cells is quite significant. The proportion of B cells with intracellular bacteria has been observed to constitute 10% of all colony-forming units from infected mice, and the B cells with intracellular bacteria isolated from infected mice transferred the disease to naïve hosts. Thus, B cells can contribute to the dissemination of infection and even to the regulation of immune response. There is indirect evidence that *Brucella*-positive B cells are the source of TGF-β1, which is known to depress the function of T cell responses and influences the course of *Brucella* infection in mice and humans [34–36].

Salmonella enterica, an intracellular bacterium, is divided into four subspecies containing many serovars. *S. enterica* serovars Typhimurium and Enteritidis are the most serious pathogens (pathovars), causing disease in a variety of animals. Serovars Typhi and Paratyphi are human-restricted pathogens causing typhoid and paratyphoid fever, respectively. *Salmonellae* are Gram-negative facultative anaerobes that infect the hosts by way of the alimentary tract. Only 0.00005% of the original infection inoculum penetrates, under conditions suitable for bacteria, through the wall of the small intestine into the organism. In general, the contact of *Salmonella* with the host cell induces a ruffling of the cell membrane, which is followed by macropinocytosis of the bacterium. Bacteria are internalized into the phagosomes that subsequently merge with endosomes and thus create the specific niche – the *Salmonella*-containing vacuole (SCV). The process of internalization into macrophages and survival inside the phagocyte is controlled by the expression of genes localized in the five *Salmonella* pathogenicity islands (SPI) coding the Type III secretion systems (T3SS), invasion proteins, and virulence genes vital for intracellular bacterium [37].

Among the cells that *Salmonella* invades are the B cells [38]. The living niche of *S. enterica* serovar Typhimurium in B cell line A20 is provided by SCVs, which can be characterized as late-endosomal/lysosomal compartment. However, the SCV environment in B cells differs from the SCV environment inside macrophages and enables the *Salmonellae* to more efficiently express the virulence genes. Even the activation of cells with IFN-γ that generally contributes to escalation of the host cell's defense does not completely applied to B cells. *Salmonella* survives more efficiently in IFN-γ activated B cells than in activated macrophage cell lines [39]. As in mouse spleen B cells and B cell lines, *Salmonella* survives in B cells, B cell precursors, and plasma cells in murine bone marrow [40]. The invasion of *Salmonella* into the enterocytes and macrophages and its entry into the B cells is an active process controlled by