REVIEW

Arch. Immunol. Ther. Exp., 2008, **56**, 77–83
PL ISSN 0004-069X

DOI 10.1007/s00005-008-0014-5

The role of B lymphocytes as antigen-presenting cells

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Received: 2007.10.30, **Accepted:** 2008.01.25, **Published online first:** 2008.03.31

Abstract

B lymphocytes are regarded as professional antigen-presenting cells (APCs) despite their primary role in humoral immunity. Over the last two decades, studies designed to define the role of the B cells as APCs have generated discrepant results, showing that B cells are either unnecessary or required for T cell priming and either immunogenic or tolerogenic to T cells. The reasons for these discrepancies are not clear. Here we review mechanisms regulating B cell antigen presentation and the data derived from the major studies conducted by different groups representing each school of thought. In general it is clear that B cells process and present specific and nonspecific antigens differently. The presentation of specific antigen through the B cell antigen receptor occurs with very high efficiency and is associated with B cell activation, resulting in the activation of cognate T cells. In contrast, the presentation of nonspecific antigen by B cells is minimized and dissociated from B cell activation. As a result, B cells inactivate T cells that recognize nonspecific antigenic epitopes presented by B cells, or they induce regulatory T cell differentiation or expansion. These mechanisms serve to ensure effective production of high-affinity antigen-specific antibodies but minimize the production of nonspecific antibodies and autoantibodies.

Key words: B lymphocytes, antigen presentation, HLA-DO, regulatory T cells.

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INTRODUCTION

B lymphocytes play an essential role in humoral immunity. In an antibody response against (T-dependent) protein antigens, B cells differentiate into memory cells or plasma cells that express or secrete high-affinity antigen-specific antibodies through a complex process involving participation of antigen-specific $CD4^+$ helper T cells [56]. Since the realization over two decades ago that B cells obtain T cell help by acting as antigen-presenting cells (APCs), the general roll of B cells as professional APCs has been vigorously investigated, re-investigated, and compared with that of the other types of APCs, such as dendritic cells (DCs) and macrophages. The conclusions from these various studies have been inconsistent. B cells are reported to be either required or unnecessary for T cell priming and either immunogenic or tolerogenic for T cells. In the present paper we review recent advances in the study of B cell antigen presentation and attempt to integrate the current understanding of this topic.

EXPRESSION OF THE COMPONENTS OF THE MHC CLASS II PATHWAY DURING B CELL MATURATION AND ACTIVATION

Like other types of professional APCs, B cells constitutively express MHC class II (MHCII) molecules, and this expression is initiated during B cell development in the bone marrow at the progenitor (pro-B) cell stage and maintained afterwards throughout B cell differentiation and maturation [13]. The potential function of MHCII in bone-marrow developing B cells (referred to as d-BCs in this article, which includes pro-B, through pre-B, to immature B cells) remains to be determined. If mature B cells (mBCs) express MHCII as a mechanism to present antigen to CD4 T cells and obtain T cell help for the production of high-affinity antibody, this cannot be applied to d-BCs since d-BCs are only in the process of rearranging their immunoglobulin (Ig) genes and are unable to make Igs. Another possibility is that B cells require MHCII expression in order to develop, differentiate, and mature. This possibility, however, is

not supported by the findings that B cell development is apparently normal in MHCII-deficient mice [48] and in patients with bare lymphocyte syndrome [59], where MHCII expression is interrupted due to loss of function of transcription factors.

In an attempt to understand the reason for the MHCII expression of d-BCs, we studied the expressions of the major components of the MHC class II antigen--processing pathway during B cell development and differentiation [15]. While d-BCs in human bone marrow express HLA-DR at a level comparable to that of mBCs, d-BCs express very little or completely lack expression of CD40, CD80, and CD86. Thus d-BCs are incapable of providing normal co-stimulation during antigen presentation to CD4 T cells consistent with a potential tolerance-inducing function. Indirect assessment of the MHCII-bound peptide repertoire presented by d-BCs, as measured by the cell surface level of the invariant chain-derived peptide CLIP, reveals that mBCs present a relatively high level CLIP. In contrast, the d-BCs present almost no CLIP on their HLA-DR molecules. The lack of CLIP-DR complexes on d-BCs suggests that all the DR molecules in these immature cells have undergone at least one round of peptide exchange to replace CLIP with other self peptides before the class II complex appears on the cell surface. The relatively high levels of CLIP-DR complexes on mBCs, on the other hand, suggest that peptide exchange activity in mBCs is so slow that at least a proportion of the DR molecules does not undergo even a single round of peptide exchange before appearing on the cell surface. The attenuation of peptide exchange in mBCs is not due to the absence of HLA-DM, a chaperon protein that facilitates peptide exchange, since a high level of DM is detected in the mBC. Rather the high level of CLIP appears to result from the expression of HLA-DO, an inhibitor of DM which is identified only in mBCs and not in d-BCs. Therefore, d-BCs, despite their immature nature, selectively express DM, but not DO, and preferentially present non-CLIP self peptides. Given their tolerogenic phenotype, we propose that the presentation of self antigens by the d-BC serves as a mechanism to induce peripheral T cell tolerance to self antigens that are uniquely presented at this stage of B cell development.

The finding that DO is expressed selectively in mBCs but not in d-BCs was unexpected. Because DO inhibits DM function, attenuating its peptide-loading activity, one might expect an increase in DM function as B cells mature so that mBCs can more effectively present antigen. There are at least two possibilities for this paradoxical finding. One is that the mBC expresses DO to diminish the presentation of endogenous self antigens. Since mBCs express CD40 and can be activated to become potent APCs with a capacity to activate pathogenic autoreactive T cells if they present high levels of the appropriate self peptides [64], the presentation of self antigens may be limited by DO expression. As a result, mBCs present more CLIP than d-BCs. A second possibility is that DO enhances the ability of the mBC to present exogenous (foreign) antigens. This possibility is suggested by the finding that only about 50–60% of the DM molecules in B cells are bound to DO, leaving about 40–60% of DM free from the DO inhibitory function [15, 36]. The amount of free DM increases when DO synthesis is increased, keeping the ratio of free DM to DO-DM complexes relatively stable [27]. When the DO-to-DM ratio is followed along the endocytic pathway, it is found that this ratio is high in the early but low in the late aspect of the pathway [31]. These findings taken together suggest the possibility that during its synthesis in the endoplasmic reticulum, all DM molecules are bound by DO, and as the DO-DM complexes travel along the endocytic pathway reaching the peptide-loading compartment in the late endocytic pathway, the complexes dissociate, with DO being degraded due to its intrinsic instability, generating free DM to catalyze peptide exchange. Therefore, due its temporal association with DO, the function of DM in the early aspect of the class II biosynthetic pathway may be selectively inhibited, and lack of DM activity at this part of the pathway eliminates or slows down the loading of endogenous self peptides on nascent, emerging MHCII. As a result, DO may prevent the emerging MHCII from forming stable complexes before reaching late endosomal compartments, keeping them accessible to subsequent peptide exchange events that ultimately result in the selection of kinetically stable peptide complexes. Lysosomes and late endosomal compartments are enriched in antigens internalized through the B cell antigen receptor (BCR). By preferential attenuation of DM-catalyzed peptide loading and exchange in the early biosynthetic pathway, DO may inhibit the presentation of endogenous high-affinity self peptides and favor the presentation of exogenous peptides that are enriched in the late endosomal pathway (Fig. 1).

Upon encountering specific antigens, B cells migrate to lymphoid follicles [29], where they undergo germinal center (GC) reactions. In the GC, B cells down-regulate DO protein expression [15, 30]. The loss of DO expression in the GC B cells appears to be through post-translational mechanisms. It is not yet clear why GC B cells need to down-regulate DO expression. Given the fact that B cells in the GC undergo somatic hyper-mutation of the Ig genes and compete for interacting with CD4 T cells for survival and antibody-affinity maturation, it is conceivable that loss of DO expression in the GC B cells serves to attenuate antigen presentation. As a result, only the B cells bearing the BCR with the highest affinity for a specific antigen might effectively compete to capture and present sufficient antigen to T cells to acquire T cell help and survival signals. As the B cells exit the GC to become memory B cells, DO expression is restored.

While further studies are needed to delineate the purpose of the regulated expression of class II MHC during B cell differentiation and maturation, the way expression is controlled suggests that B cells regulate their MHCII expression to meet antigen presentation

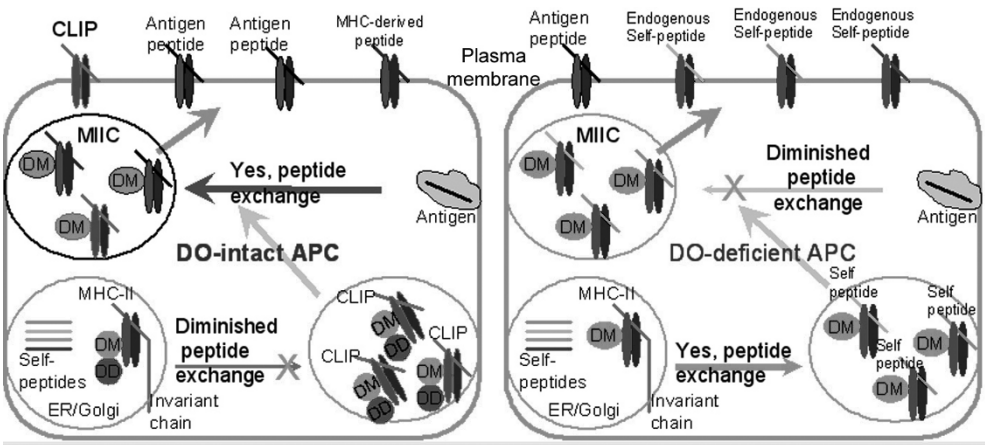

Fig. 1. Model for antigen presentation in the presence or absence of HLA-DO. In the presence of DO (left), APC efficiently present foreign peptides or self peptides derived from membrane or secretory proteins that are localized in late endosomal compartments (MIIC, MHCII compartment). In the absence of DO, endogenous peptides derived in the early aspect of the secretory and endocytic pathways are presented dominantly. ER – endoplasmic reticulum.

requirements that are distinct for each stage of differentiation and maturation.

B CELL ANTIGEN PROCESSING

It is often believed that antigens presented through the class II pathway are typically of exogenous origin, including extracellular proteins, or they are derived from membrane proteins or proteins of the secretory pathway that gain access to endosomal compartments. Analysis of peptides eluted from MHC class II molecules, however, identified a substantial fraction of peptides (up to 20%) derived from cytosolic and nuclear proteins [17, 21, 23, 51]. Autophagy appears to constitute a major mechanism for the delivery of endogenous proteins to the class II pathway for presentation [63]. While the general impact of the presentation of the endogenous peptides remains to be learned, the quantity of a given self peptide is typically very low, and weak stimulation with self peptides may contribute to the induction of T cell anergy [64].

Exogenous proteins gain access to B cells through a receptor-independent mechanism, such as fluid phase pinocytosis, or specifically, through receptor-mediated mechanisms such as BCR-mediated endocytosis [5]. Although both mechanisms can generate quantities of peptide-MHCII complexes sufficient for T cell activation [72], BCR-mediated presentation of specific antigen is far more efficient (as much as 100–10,000 fold) than the presentation of pinocytosed antigens [42]. *In vitro*, B cells bearing BCRs with an average affinity of $\sim 10^{-8}$ M are capable of presenting the antigen at concentrations of 10^{-11} M, where only a very small fraction of BCR (~0.05%) is occupied by antigen [40]. Upon immunization, antigen-specific B cells, but not DCs or nonspecific B cells, are the first APCs in the lymph node (LN) to acquire antigen and express the specific MHC-peptide complexes [8, 54], highlighting the capacity of B cells to efficiently capture and present BCR-internalized antigen *in vivo*.

Multiple mechanisms contribute to the extraordinarily efficiency of BCR-mediated antigen presentation. First, the high-affinity BCR enables B cells to efficiently capture specific antigens present even at extremely low but immunologically relevant concentrations, far below those required for presentation by antigen-nonspecific B cells as well as DCs [41, 54]. Secondly, BCR cross-linking activates BCR-Igα/Igβ signaling, unleashing a cascade of downstream intercalated biochemical events that ultimately lead to B cell activation [9, 18]. As a consequence, multiple mechanisms and pathways become activated. For instance, formation of the antigen-BCR complex induces ubiquitination of the IgM heavy chain [24] and the translocation of BCR-antigen complexes to lipid rafts, leading the BCR-antigen complexes to undergo accelerated transport to late endosomal compartments [7, 16, 68]. BCR signaling also induces the reorganization, fusion, and acidification of Lamp1-positive late endosomes where class II MHC molecules and invariant chain accumulate [65], forming new multivesicular bodies into which DM is recruited [39]. The process involves the activation of phosphoinositide 3-kinase [2, 32]. The internalized antigen-BCR complexes persist for prolonged periods of time within late endocytic compartments enriched in DM but deprived of DO [31]. Therefore, not only is BCR-mediated antigen processing more efficient, but also functionally distinct. These properties enable B cells to optimally activate cognate T cells, thus promoting survival and activation of cognate B cells [52]. Furthermore, BCR-mediated but not nonspecific antigen presentation induces B cell activation through CD40 interaction with CD40 ligand expressed on activated cognate T cells [53], which further augments the co-stimulatory function of B cells [1, 19, 22].

Taken together, owing to their inefficiency in capturing and processing pinocytosed or endogenous antigens and their inability to provide co-stimulation prior to activation, resting B cells are indolent APCs for the presentation of nonspecific antigens compared with other types of professional APCs, such as DCs and macrophages. However, upon encountering specific antigens through their BCR, B cells become potent

APCs capable of presenting specific antigenic determinants with extremely high efficiency. Therefore the signals delivered to T cells by resting and BCR-activated B cells differ not only in quantity, but also in quality. These mechanisms provide the basics for understanding the different outcomes of B cell-mediated antigen presentation.

THE OUTCOME OF B CELL ANTIGEN PRESENTATION

As soon as the role of MHCII in T cell recognition of antigen was recognized, B cells were tested *in vitro* for their ability to present antigen. Only activated, but not resting, B cells were initially found to be able to present antigens [4]. It was soon realized, however, that the inability of resting B cells to present antigen was due to the high-dose irradiation they routinely received under common experimental protocols, and that resting B cells were highly radiation sensitive. Non-irradiated small B cells presented antigens very well [3]. In our laboratory, resting nonspecific B cells can effectively present 10–50 μg/ml concentrations of ovalbumin to naïve ovalbumin-specific OT-II transgenic T cells and induce robust T cell proliferation. It should be noted, however, that the ability of B cells to induce T cell proliferation *in vitro* does not necessarily signify that the B cells will activate T cells in an immunogenic manner *in vivo*.

To examine the role of B cells as APCs *in vivo*, B cell-depleted mice (via administration of anti-μ antibody) were immunized with different protein antigens in complete adjuvant (complete Freund's adjuvant). Approximately one week post-immunization, T cell--enriched or whole draining LN cells were re-stimulated *in vitro* with the corresponding antigens. It was observed that the LN cells of the B cell-deprived mice proliferated poorly compared with those of the B cell-intact mice, as measured by thymidine incorporation [33, 34]. When the mice were reconstituted with B cells prior to immunization, their LN cells responded well to antigen restimulation [37, 60]. These results led to the conclusion that B cells were required for optimal priming of T cells *in vivo*. This conclusion was soon challenged by subsequent studies [25, 57, 61, 66, 67]. Using SCID mice reconstituted with T cells, with or without B cells, or B cell-knockout (μMT) mice, studies conducted by multiple groups demonstrated that T cells could be successfully primed with various antigens *in vivo* in the absence of B cells, and the data were convincing. The conclusion that B cells are not required for T cell priming *in vivo* was further supported by additional observations. For example, in the experimental autoimmune encephalomyelitis (EAE), a disease mediated by CD4 T cells, disease progression and severity were not attenuated by the absence of B cells [70]. B cell-deficient mice immunized with cardiac myosin developed myocarditis comparable in incidence and severity to that induced in wild-type mice [49].

In order to understand the reasons for the major discrepancy in the conclusions from these various studies, we have carefully reviewed the relevant experimental procedures and identified a potential unrecognized pitfall associated with the experiments performed in the initial studies. In those experiments, *in vitro* re-stimulation was performed by adding antigen to unfractionated LN or nylon wool or anti-Ig panned T cell-enriched LN cells without the addition of exogenous APCs. As a result, the cellular components of the cultures differed between the relevant experimental groups. The normal mouse LN cell cultures would have contained many antigen-specific B cells, but the B cell-deprived mouse cells would not. T cells enrichment by nylon wool or anti-Ig panning procedures can still contain a significant fraction of residual B cells, up to 20–45% of the total cells based on our own experience. The antigen-specific B cells are derived from GC reactions that peak at 7–10 days post-immunization since nearly all the GC B cells are antigen-specific [45]. These B cells might function not only as highly potent APCs (as describe above) to induce T cell proliferation, but also as responder cells to undergo vigorous proliferation in cultures, giving rise to a high proliferation index. In LN from B cell-depleted mice, however, B cells were absent, and other types of APCs, such as DCs or macrophages, could have been lost or present at very low numbers due to manipulation, such as panning, or when collagenase was not used. It is therefore possible that the diminished T cell proliferation observed in cultures of cells from B cell-depleted mice was due to a relative lack of APCs, especially antigen-specific B cells, but not to a lack of responding antigen-specific T cells.

Nevertheless, do B cells really have no impact on T cell priming? The debate continues as new lines of evidence emerge. It was reported that during antigen--specific T-B cell interaction in normal B6 mice, the induction of optimal T cell priming to a protein antigen requires the involvement of antigen-specific B cells [19]. A study of B cell-deficient mice generated by disruption of the Ig-Jh gene reveals that antigen-primed T cells from the B cell-deficient mice were incapable of providing help to B cells [47]. Other studies suggest that the development of memory CD4 cells is impaired in B cell--deficient mice [20, 44]. B cells are also reported to be required for the generation of protective effector and memory CD4 cells in response to pneumocystis lung infection [46]. μMT mice retain a predominant $CD4^+$ Th1-like response to malarial antigens throughout a primary infection [38]. Furthermore, induction of autoreactive B cells allows for priming of specific autoreactive T cells [43], and the development of spontaneous autoimmune thyroiditis in NOD.H-2h4 mice requires B cells [6]. In the murine models of lupus, nuclear antigen-specific B cells, but not the autoantiboies, are essential for disease expression [10], and antigen-specific B cells are responsible for driving early T cell autoimmunity *in vivo* prior to DC-mediated autoantigen presentation [71], a finding supported by a very recent study

[54]. Therefore, although not absolutely required, B cells play a definite significant role in T cell priming, positively impacting the function of T cells.

Additional evidence is available implicating a role for B cell antigen presentation in tolerance. When resting B cells from male mice were transferred to syngeneic female mice, CD8 T cells specific for male H-Y antigen in recipient mice became inactivated and eventually disappeared. To determine whether the tolerogenic activity was only associated with resting B cells, the B cells was activated with lipopolysaccharide (LPS) before being transferred to the recipients and the same outcome was observed [28]. Similarly, transcriptional targeting of antigen to B cells is reported to induce antigen-specific CD8 T cell tolerance [69]. In the EAE model, infusion of autologous B cells expressing a transgene-encoded encephalitogenic determinant induced antigen-specific unresponsiveness and protected mice from the induction as well as relapse of EAE through the induction of CD4 T cell tolerance [11, 12]. B cells were also reported to inhibit graft-versus-host disease [62]. While the mechanism responsible for B cell--induced tolerance has yet to be fully characterized, it may involve T cell anergy or deletion. Several studies have suggested a role for B cells in the induction of regulatory T cells [50, 58]. We have recently found that B cells preferentially induce proliferation of FoxP3$^+$ regulatory CD4 T cells *in vitro*, providing direct evidence for this idea [14].

Apparently, the studies described above demonstrate that B lymphocytes as APCs can impact T cell function both in a positive as well as a negative manner, leading to T cell activation or tolerance. The question, then, is what makes the same cells immunogenic under some circumstances but tolerogenic under others? Examining the nature of the antigens involved in the various experiments described above reveals that B cells promote immunity when presenting specific antigens (with one exception [26]), but they induce tolerance when presenting nonspecific antigens, consistent with the rules governing B cell antigen presentation as proposed above. B cells become activated and potent APCs as they encounter specific antigens, bringing about a cognate T-B cell interaction leading to T cell activation and GC reactions. The exception is with a monovalent specific antigen [53], which does not activate B cells or enhance antigen presentation because of the inability to cross-link BCRs [35]. On the other hand, the presentation of nonspecific antigens, such as endogenous H-Y or EAE-related antigens, does not result in B cell activation. Under these conditions, the outcome is T cell tolerance. Are B cells which are activated nonspecifically during infections capable of activating self reactive pathogenic T cells by the presentation of nonspecific self antigens? The answer seems to be no. B cells activated by LPS, but not by anti-Ig and anti-CD40, induce CD8 T cell anergy mediated by transforming growth factor β [55], consistent with the findings made in a previous study [28]. In addition to the lack of B cell activation, the low level of peptide determinants associated with the presentation of nonspecific antigens might also contribute to the tolerogenicity of B cells. As shown in a recent study, B cells constitutively present a low level of endogenous myelin basic protein (MBP) and induce tolerance in MBP-specific T cells. However, when the dose of MBP epitopes presented was increased by adding exogenous MBP peptide, the B cells started activating T cells [64]. Similarly, as we have found, although B cells preferentially induce the proliferation of allogeneic FoxP3$^+$ regulatory T cells at a B-to-T ratio of 1:1 or lower, a high B-to-T cell ratio completely abrogates this tolerogenic propensity [14].

In conclusion, as the primary player in humoral immunity, B cells present specific antigens to cognate CD4 T cells with extremely high efficiency so that they obtain help for the production of high-affinity antibodies. Nonspecific antigens derived from endogenous and pinocytosed proteins are also presented by B cells, but the outcome of presentation of nonspecific antigens is T cell tolerance. Therefore, B cells can either activate or inactivate T cells, depending on the nature of the antigen. In the presence of DCs or activated macrophages, the role of B cells in presenting nonspecific antigens is negligible. However, if B cells are the only cells available to present nonspecific antigen, T cell tolerance would be the outcome. Such differences in antigen presentation between antigen-specific and nonspecific B lymphocytes provide assurance that specific T-helper cells will be the favorite partners for cognate interactions with specific B cells, a mechanism that is essential not only for the effective induction of specific antibody responses, but also for the prevention of non-specific humoral immunity. Many questions remain to be addressed. For example, is the presentation of specific antigens by B cells in the absence of DCs sufficient to prime CD4 T cells? What exact role do B cells play *in vivo* in the maintenance of FoxP3$^+$ regulatory T cells? Similarly, some hypotheses described in this paper need further experimental evaluation, including a critical evaluation of the function of MHCII expressed in d-BCs as well as the reasons for the loss of DO expression in GC B cells.

Acknowledgment: This work was supported by grants from the United States National Institute of Allergy and Infectious Diseases, National Institutes of Health.

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