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Complement Opsonization Is Required for Presentation of Immune Complexes by Resting Peripheral Blood B Cells

Susan A. Boackle,* Margaret A. Morris,* V. Michael Holers,* and David R. Karp†

Complement receptor 2 (CD21, CR2) is a B cell receptor for complement degradation products bound to Ag or immune complexes. The role of CD21 in mediating Ag presentation of soluble immune complexes by resting B cells was studied. Complement-coated immune complexes were formed by the incubation of influenza virus with serum from immune donors. These complexes bound to peripheral blood B cells in a complement-dependent manner. The binding required CD21 or, to a lesser extent, complement receptor 1 (CR1, CD35). B cells pulsed with immune complexes containing complement elicited a response from a panel of influenza-specific T cell clones, while those pulsed with immune complexes formed in the absence of complement did not. The expression of the early activation marker CD69 and the costimulatory molecule CD86 were not induced by CD21 ligation alone, suggesting that CD21-mediated Ag presentation occurs independently of B cell activation. Up-regulation of these markers required exposure to T cell factors elicited by the recognition of Ag derived from complement-containing immune complexes. These findings suggest that binding of Ag to CD21 enables Ag-nonspecific B cells to participate in the activation of Ag-specific T cells in a process that occurs independently of well-characterized B cell activation events. The Journal of Immunology, 1998, 161: 6537–6543.

The development of a mature immune response requires the collaboration between B and T lymphocytes. Ag is internalized by B cells and processed into peptides that are presented to T cells in the context of specific MHC class II molecules. Ag-specific T cells recognize these complexes via TCR/CD3 with signals through these receptors resulting in the up-regulation of CD40 ligand on the T cell surface. Cross-linking of B cell CD40 by CD40 ligand induces the expression of the costimulatory molecules CD80 and CD86 (1, 2), which bind CD28 on T cells. The T cells then secrete IL-2, which enhances B cell maturation and proliferation. An individual B cell is able to initiate these responses only if it presents adequate numbers of class II peptide complexes to specific T cells. Although nonspecific fluid-phase endocytosis of Ag occurs poorly in resting B cells, Ag can be internalized efficiently by binding to specific Ag receptors (3). Nonetheless, B cells are not believed to be critical for Ag presentation because of the low frequency of Ag-specific B cells for any particular Ag.

Ag can be targeted to other B cell surface receptors, including complement receptors such as CD21. CD21 is a 140-kDa glycoprotein located on the surface of mature B lymphocytes, as well as on follicular dendritic cells, pharyngeal epithelial cells, thymocytes, and some T cells. It binds the terminal products of C3 degradation that have become covalently bound to Ag or immune complexes in the course of complement activation. Studies performed in vivo have confirmed the critical role of CD21 in the generation of a normal humoral immune response. Normal murine Ab responses to T-dependent Ags are inhibited by pretreatment with mAbs to CD21 or soluble CD21 (4–6). Mice rendered CD21-deficient using gene-targeting techniques demonstrate similar alterations (7, 8). B cell CD21 was found to be critical for these effects based on studies in bone marrow chimeras, in which the immune defects were corrected by reconstitution with bone marrow from CR2+/+ MHC-matched littersmates. The role of B cell CD21 was further confirmed in mice created by RAG-2-deficient blastocyst complementation (9). These animals specifically lack CD21 on their B cells, but have normal expression on their follicular dendritic cells (FDC). These studies demonstrated that the targeting of complement-coated immune complexes to FDC is not sufficient for the development of a normal humoral response in the absence of complement receptors on B lymphocytes. Additional studies have supported an adjuvant-like role of complement in B cell responses. C3d linked to hen egg lysozyme (HEL)3 by recombinant DNA techniques was 1,000–10,000 times more immunogenic than HEL alone, inducing enhanced C5a2+ mobilization in vitro as well as augmenting primary and secondary Ab responses in vivo (10).

CD21 may participate in the development of a normal immune response by several mechanisms. First, ligation of CD21 may result in various signals that are critical for normal B cell responses. Previous studies have shown that B cell proliferation and differentiation can be induced by cross-linking CD21 with polymeric C3d or certain anti-CD21 mAbs in the presence of T cell factors (11, 12) or phorbol esters (13). CD21 also plays a synergistic role in the activation of B cells induced by ligation of surface IgM, as measured by calcium mobilization (14), proliferation (15), or induction of c-fos mRNA levels (16). Many of the signaling effects induced by CD21 have been attributed to its physical association with CD19 and CD81 (TAPA-1) on the surface of B lymphocytes in vivo (17).

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3 Abbreviations used in this paper: HEL, hen egg lysozyme; KLH, keyhole limpet hemocyanin; PE, phycoerythrin; CR, complement receptor; TAPA-1, target of anti-proliferative antibodies-1.
Materials and Methods

Antibodies

Polyclonal goat Abs to influenza hemagglutinin (A/Aichi/2/68) were obtained from the National Institute of Allergy and Infectious Diseases Biological Reagent and Reference Repository (anti-H; Braton Biotech, Rockville, MD). The murine mAbs to CD21 (HB5), CD3 (OKT3), CD4 (63D3), CD16 (B73.1), and CD11b (LM2/1.6.11) were obtained from the American Type Culture Collection (Manassas, VA). Phycoerythrin (PE)-labeled anti-CD19 (B43) was obtained from Pharmingen (San Diego, CA). Biotinylated anti-CD19 (B4) was obtained from Coulter (Miami, FL). FITC-labeled anti-CD69 was obtained from Becton Dickinson (Sunnyvale, CA). FITC-labeled CD86 was obtained from The Binding Site (Birmingham, U.K.). Rabbit polyclonal Ab to CD21, Ab73, was generated by immunization with a soluble human CD21 produced by recombinant baculovirus in insect cell culture. It recognizes short consensus repeats 1–2 of CD21 including the C3d binding site (V.M.H., unpublished data). The mouse mAb to CD32, AT10 (26), was the kind gift of Dr. P. M. Morganelli (Veterans Administration Hospital, White River Junction, VT). The mouse mAb to CD35, 3D9, was the kind gift of Dr. Eric Brown (Washington University, St. Louis, MO) (27).

Purification of peripheral blood B lymphocytes

B cells were purified from peripheral blood by either negative or positive selection. For negative selection, PBMCs were depleted of monocytes and NK cells by treatment with 0.09 mg/ml leucine methyl ester for 45 min at room temperature, and subsequently depleted of T cells by rosetting with SRBCs. The remaining population was treated with 10 μg/ml OKT3, 63D3, B73.1, and LM2/1.6.11, followed by labeling with goat anti-mouse magnetic beads (MACS; Miltenyi Biotec, Sunnyvale, CA), and selection for negative cells from a high-gradient magnetic separation column (MACS; Miltenyi Biotec). For positive selection, PBMCs were stained with biotinylated mAb to CD19 (B4) and selected over a Cellprep LC avidin column (Cellpro, Bothell, WA). Cells were analyzed after purification by staining with PE-labeled anti-CD19 (B43; Pharmingen, San Diego, CA) and were determined to be 90–98% CD19-positive. The resting state of these cells was confirmed by their lack of expression of CD69 and CD86.

Preparation of immune complexes

Influenza virus HK X-31 (recombinant between A/Aichi/2/68 and A/PR/8/34) was grown in embryonated chicken eggs and live virus purified over a sucrose gradient. Virus was inactivated by UV irradiation. Immune complexes were generated by incubation of inactivated virus with RPMI 1640 and 5% normal human serum from a single donor which contains high titers of influenza-specific IgG (24). Complexes were allowed to form at 37°C for 1 h. Serum samples that were inactivated by heat (56°C for 1 h) or by incubation with methylamine (Sigma, St. Louis, MO) (100 mM, 37°C for 1 h followed by exhaustive dialysis against PBS) were used to generate immune complexes lacking complement. In some experiments, synthetic immune complexes were generated by incubating biotinylated human IgG1 (Sigma) with FITC-streptavidin (Life Technologies, Gaithersburg, MD) for 30 min at 4°C. Either normal human serum or methylamine-inactivated human serum was added to a final concentration of 5% and complement activation allowed to occur at 37°C for 1 h.

Microcytotoxicity assays to analyze immune complex binding

Immune complexes were bound to cells for 30 min at 37°C then washed twice with PBS. They were then analyzed by microcytotoxicity on a FACScan (Becton Dickinson, Sunnyvale, CA). For blocking experiments, blocking Abs were incubated with cells for 30 min at 4°C before the addition of immune complexes. Immune complex binding was detected using goat anti-FITC serum followed by FITC-sheep anti-goat IgG in the case of the influenza-containing complexes, and directly with the IgG/FITC-streptavidin complexes. In experiments involving PBMC populations, B cells were labeled with PE-labeled CD19, and histograms were generated by gating on the CD19-positive cells.

Generation of T cell clones

A panel of influenza-specific human T cell clones was generated from a donor who possesses the MHC class II haplotype: DRB1*0101/DRB1*0401, DRB1*0501/DRB1*0301 (24). T cells were characterized for MHC class II restriction and Ag specificity by testing their IL-2 response to influenza virus or peptide presented by B cell lines and PBMC of known haplotypes (S.A.B., unpublished data). MHC class II restrictions were confirmed by blocking T cell responses with mAbs to class II molecules. Characterization of TCR was performed as described (28). CL19 is restricted by HLA-DR1 and -DR4/Dw4, CL23 and CL58 by HLA-DR1, CL35 by HLA-DQ1, and CL62 by HLA-DRw53. CL19 and CL58 both recognize the immunodominant peptide of hemagglutinin, HA307-319.
CL62 recognizes a peptide contained within the influenza nucleoprotein. Identification of the peptide Ags for the other clones has not been completed.

**Ag presentation assays**

Highly purified peripheral blood B cells or B cell lines were incubated with complement-influenza immune complexes for 4 h at 37°C, washed to remove free immune complexes, then plated with T cells in a 96-well round bottom plate at stimulator:responder ratios from 2:1 to 5:1. T cell clones were washed twice to remove excess IL-2 before plating. In some experiments, peripheral blood B cells were incubated for an additional 20 h before addition of T cells. As shown below, this additional incubation period was found to be critical for the presentation of immune complexes by resting B cells. To test whether Ag processing occurred before contact with T cells, the resting peripheral blood B cells were first incubated with immune complexes for 4 h, washed, then incubated for 20 h at 37°C. The cells were then fixed with 0.75% paraformaldehyde in serum-free medium for 5 min at 37°C and then for 1–2 h at 4°C. Ten percent FCS in PBS was added to quench the fixative, and the cells were washed and plated with T cells. In all cases, T cell supernatants were harvested after 24 h at 37°C and tested for IL-2 by CTLL-2 bioassay as described (29).

**Microcytofluorometry assay to analyze B cell activation and up-regulation of costimulatory molecules**

Peripheral blood B lymphocytes were incubated with immune complexes for 4 h, washed, and plated in 96-well round bottom plates at 20,000 cells/well in RPMI 1640 containing 10% heat inactivated human serum (HIHS). After 20 h, T cell clones were added at 20,000 cells/well. Cells were harvested after 24 h, stained with PE-labeled anti-CD19 and FITC-labeled anti-CD69 or CD86, and analyzed by dual color microcytofluorometry. Histograms were generated by gating on the CD19-positive cells.

**Results**

**Complement-influenza immune complexes bind to peripheral blood B cells in a complement- and CR-dependent manner**

We have previously demonstrated that immune complexes formed under physiological conditions bind to CD21-transfected fibroblasts and to transformed B cells in a complement- and CD21-dependent fashion (24). The following experiments were performed to confirm that binding of these complexes to peripheral blood B cells occurs in a similar manner. Immune complexes were formed from either acquired anti-influenza IgG and purified virus, or biotinylated IgG and streptavidin. The complexes were exposed to normal or heat- or methylamine-treated serum then were incubated with PBMCs, and binding to CD19-positive cells was analyzed by microcytofluorometry. Binding was complement-dependent, as it did not occur with immune complexes formed in serum in which complement had been inactivated (Fig. 1). Identical results were obtained whether the complexes were formed between influenza virus and acquired Abs, or between biotinylated IgG and streptavidin (data not shown).

Additional experiments were performed to identify the specific receptors involved in the binding of immune complexes to peripheral blood B cells. These cells express moderately high levels of the B cell C3 receptors, CD21 and CD35, in addition to the B cell FcyRII, CD32, and little, if any, complement receptor 3 (CD11b/CD18; CR3) (data not shown). Blocking the binding domains of either CD35 (CR1) or CD21 alone partially inhibited binding, whereas blocking both CD21 and CD35 simultaneously completely abrogated immune complex binding (Fig. 2). There was no effect on immune complex binding when the cells were pre-incubated with a blocking mAb to CR3 or CD32, nor could anti-CD32 enhance the inhibition of complex binding seen with anti-CD21 alone (data not shown). These data suggest that fluid-phase activation of complement by soluble immune complexes generates ligands for both CD35 (C3b and C4b) and CD21 (presumably iC3b). One particle may have either or both types of ligand. Binding of complement-containing immune complexes to both complement receptors was seen, whereas no binding to the B cell Fc receptor could be demonstrated in mixtures prepared in serum in which complement was inactivated. Again, identical data were obtained regardless of the type of immune complex used.

![Figure 1](http://www.jimmunol.org/) Binding of immune complexes to peripheral blood B cells occurs in a complement-dependent manner. Influenza-containing immune complexes formed in either normal human serum (thick line) or heat-inactivated human serum (thin line) were incubated with highly purified peripheral blood B cells. Binding of immune complexes was detected by staining with anti-H3 Ab, which is specific for influenza hemagglutinin. Peripheral blood B cells bound high levels of complement-containing immune complexes and did not bind immune complexes that lacked complement. The background fluorescence of cells not incubated with immune complexes is indicated by the dotted line.

![Figure 2](http://www.jimmunol.org/) Immune complexes bind to CD21 and CD35 on peripheral blood B cells. Peripheral blood B cells were treated with medium alone (A) or with blocking Abs to CR1 (3D9) (B), CR2 (Ab73) (C), CR3 (LM2/1.6.11) (D), CD32 (AT10) (E), or CR1 plus CR2 (F) before incubation with complement-containing immune complexes (thick line). Fluorescence of cells treated with isotype-matched species-specific control Abs before the addition of immune complexes is indicated by the thin line. B cells were labeled with PE-labeled anti-CD19 and histograms were generated by gating on CD19-positive cells.
Peripheral blood B cells present Ag from complement-containing immune complexes in a C<sub>9</sub>-dependent manner

We and others have shown that CD21 can participate in the presentation of Ag from complement-containing immune complexes by transformed B cells. These cells constitutively express the costimulatory molecules CD80 and CD86 and are similar to cells that have been triggered by B cell receptor cross-linking (30). We wished to determine whether resting peripheral blood B cells have a similar capacity for Ag presentation via CD21. Although resting B cells are not thought to be good APCs, ligation of CD21 may augment this ability by enhancing uptake or processing of immune complexes or by providing signals which improve the capacity of the cells to present Ag.

A panel of influenza-specific T cell clones was used to analyze the ability of resting B cells to present Ag derived from complement-coated immune complexes. Resting B cells purified from the peripheral blood of HLA-matched donors were pulsed with complement-influenza immune complexes for 4 h, then incubated for an additional 20 h before addition of the T cells. The T cell response was analyzed by measurement of IL-2 in the cell supernatants after a 24-h incubation. Significant T cell activation was seen only in response to B cells that were pulsed with immune complexes containing complement, and not to B cells pulsed with immune complexes prepared in the absence of complement (Fig. 3). Fixed B cells were used to determine whether Ag uptake and processing occurred during the period when the B cells were cultured alone, or only after coculture with the T cell clones (Fig. 4). When the B cells were fixed before addition of the T cell clones, their ability to activate the T cells was reduced but not ablated. Fixed B cells that had been pulsed with HA307-319 peptide also had reduced ability to activate the HA307-319-specific T cell clone, CL58, suggesting that fixation itself decreases the effectiveness of B cells to act as APCs. The fixation did not cause the B cells to become nonspecific stimulators of the T cell clones as CL62 did not produce IL-2 in response to the peptide-pulsed B cells.

All five of the T cell clones demonstrated an augmented response to Ag derived from complement-influenza immune complexes. It is unlikely that this is due to transfer of immune complexes or processed peptide to T cells for autopresentation rather than direct processing and presentation by B cells. The T cell clones were shown to be negative for CR1, -2, and -3, and thus would not be expected to bind intact immune complexes (data not shown). Furthermore, B cells from HLA-unmatched donors that were pulsed with complement-containing immune complexes were unable to transfer Ag to the T cell clones (data not shown). These data suggest that resting B cells are directly involved in the presentation of Ag that has bound to the cell surface via CD21.

Peripheral blood B cells are not activated by ligation of CD21

To further analyze the events that occur in the B cell subsequent to immune complex binding, the induction of the early activation marker CD69 and the costimulatory molecule CD86 was measured. Resting B cells were purified from peripheral blood. The resting state of these cells was confirmed by their lack of expression of CD69 and CD86 at time 0 (data not shown). The cells were pulsed with immune complexes for 4 h and then allowed to incubate at 37°C for an additional 20–44 h. Cells were harvested and stained with FITC-labeled anti-CD69 or anti-CD86. There was little or no evidence of B cell activation after a 48-h exposure to immune complexes as measured by CD69 expression, or of costimulatory molecule up-regulation as measured by CD86 expression (Fig. 5). In addition, there was no change in the levels of the adhesion molecules CD11a/CD18 (LFA-1) or CD58 (LFA-3) or in the costimulatory molecule CD80 on the B cells (data not shown). Similar results were found with cells harvested 20 h after being pulsed with immune complexes. These findings suggest that CD21 ligation by immune complexes does not result in activation of the B cell as measured by traditional markers.

T cell signals are required for B cell activation and induction of costimulatory molecules

Additional experiments were performed to determine the conditions required for the induction of activation markers and costimulatory molecules in this system. Peripheral blood B cells were pulsed with complement-influenza immune complexes for 4 h and allowed to incubate an additional 20 h before the addition of HLA-matched influenza-specific T cell clones to the culture. Cells were harvested after 24 h, stained with anti-CD19 and either anti-CD69 or anti-CD86, and analyzed by dual color microcytotoxicity. Some cells were pulsed with HA307-319 peptide as a positive
and were incubated with medium alone (immune complex-pulsed peripheral blood B cells. Peripheral blood B cells

FIGURE 6. Effect of T cells on the expression of CD69 and CD86 by their surface (Fig. 6). This was in contrast to those cells that had expressed high levels of CD69 and moderate levels of CD86 on complexes or HA307-319 peptide before incubation with T cells activation. The B cells that had been pulsed with either immune control for T cell activation and its reciprocal effects on B cell activation. The B cells that had been pulsed with either immune complexes or HA307-319 peptide before incubation with T cells expressed high levels of CD69 and moderate levels of CD86 on their surface (Fig. 6). This was in contrast to those cells that had been incubated in medium alone, in that few cells in this popula-

FIGURE 5. CD69 and CD86 expression on B cells pulsed with complement-containing immune complexes. Resting peripheral blood B cells were incubated with media alone (A and C) or with immune complexes prepared in the presence of complement (B and D). Cells were harvested after 24 h, stained with FITC-labeled mouse mAbs to CD69 (A and B) or CD86 (C and D) (thick lines) and analyzed by flow cytometry. Control staining with isotype matched mAb is indicated by the thin lines.

tion expressed CD69 and none expressed CD86. These data suggest that T cell factors are critical in the full activation of resting peripheral blood B cells, and that these T cell responses are elicited by the recognition of Ag derived from complement-containing immune complexes in the absence of B cell activation.

Discussion

These experiments demonstrate that resting polyclonal B cells are capable of participating in Ag presentation. By binding complement-containing immune complexes to the B cell complement receptors Ag can be targeted to all peripheral blood B cells. Although both CD21 and CD35 participate in binding of immune complexes to the B cell, CD21 likely plays the major role in immune complex internalization. Polymeric C3d, the specific ligand for CD21, can be internalized (31), whereas CD35 has not been shown to undergo endocytosis. Furthermore, Raji B cells, which express CD21 in the absence of CD35, are capable of presenting immune complex-derived Ag to T cell clones (24). Since CD35 is a cofactor for the conversion of C3b to iC3b and iC3b to C3d, immune complex bound to this receptor on the surface of the rest-

These experiments demonstrate that resting polyclonal B cells are capable of presenting complement-bound Ag to a panel of class II-restricted Ag-specific T cell clones. Interestingly, a longer incubation period before exposure to T cells was required for Ag presentation in resting B cells as compared with transformed B cells. Critical costimulatory molecules expressed constitutively on transformed B cells may need to be up-regulated on resting B cells before they can function as competent APCs. This is an unlikely explanation in this system as the up-regulation of classical costimulatory molecules (CD80/86 and LFA-1/LFA-3) was not induced by incubation with complement-containing complexes, nor do the T cell clones used in these experiments require these molecules for their activation (data not shown). Alternatively, the ability of resting B cells to internalize and process Ag may be less efficient than in transformed B cells. The B cells clearly had processed Ag and targeted specific peptide to their class II molecules in the absence of T cell help, since fixation of the cells after exposure to immune complexes did not ablate their ability to activate the T cells.

Resting peripheral blood B lymphocytes were fully capable of presenting complement-bound Ag to a panel of class II-restricted Ag-specific T cell clones. Interestingly, a longer incubation period before exposure to T cells was required for Ag presentation in resting B cells as compared with transformed B cells. Critical costimulatory molecules expressed constitutively on transformed B cells may need to be up-regulated on resting B cells before they can function as competent APCs. This is an unlikely explanation in this system as the up-regulation of classical costimulatory molecules (CD80/86 and LFA-1/LFA-3) was not induced by incubation with complement-containing complexes, nor do the T cell clones used in these experiments require these molecules for their activation (data not shown). Alternatively, the ability of resting B cells to internalize and process Ag may be less efficient than in transformed B cells. The B cells clearly had processed Ag and targeted specific peptide to their class II molecules in the absence of T cell help, since fixation of the cells after exposure to immune complexes did not ablate their ability to activate the T cells.

The physiological immune complexes formed in these experiments contain acquired anti-influenza IgG. While these data support the role of complement opsonization in B cell Ag presentation following an established humoral immune response, it is likely that other immune complexes can be presented in this manner. Natural Abs directed against a variety of foreign particles are present constitutively and can activate the classical pathway. Furthermore, the alternative pathway of complement activation can be initiated by many microbial pathogens in the absence of specific Ab. We have shown that influenza is opsonized by complement and binds equally well to B cells regardless of whether the complexes are formed by the alternative or the classical pathway of complement activation (data not shown). Since complement-opsonized complexes can be formed in the absence of high titer-specific Ig, CD21-mediated Ag presentation may also play a role in the responses to Ags for which a humoral immune response has not yet been mounted.

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Our studies demonstrate that polyclonal B cells participate in Ag presentation in vitro; a recent study using a novel mAb specific for peptide-class II complexes suggests that this process also occurs in vivo (33). After i.v. injection of high titers of soluble HEL, nearly all B cells in lymphoid tissues were found to have HEL 46-61-I-Ak complexes on their surface using a mAb specific for this complex. The mechanism by which these B cells load class II molecules with peptide was not determined, nor was it clarified whether lower amounts of injected Ag resulted in display of peptide by the majority of B cells. Nonetheless, these studies suggested that polyclonal B cells are a major player in the in vivo presentation of Ag, and it is possible that one way in which they participate in this process is by uptake of Ag-C3d complexes by CD21.

The recruitment of T cell help by Ag-nonspecific B cells could result in the polyclonal expansion of B cells and the production of nonspecific Abs. To maintain selectivity of B cell responses, the ability of the B cell to respond to T cell help may be dependent upon coligation of surface Ig and CD21. This is supported by a recent report by Thornton, et al. (34), in which the incubation of PBMC with complement-coated KLH immune complexes resulted in the synthesis of Abs specific for KLH, rather than a polyclonal Ab response. This finding suggests that although CD21 can serve as a vehicle for internalizing Ag, it will not transduce signals which result in the independent production of Ab in the absence of signals through sIg.

The ability of B cells to present Ag in different circumstances is modeled in Fig. 7. Resting B cells of diverse antigenic specificities can take up Ag by CD21. Since they do not express costimulatory molecules, as demonstrated by our studies, they are not involved in the initial activation of naive T cells (top row). However, they may be involved in the continued activation of T cells that have been previously primed by dendritic cells or activated monocytes (bottom row). Previously activated T cells are believed to have less stringent requirements for costimulation. These T cells are initially activated in the periarteriolar lymphoid sheath (PALS) region of the spleen or the parafollicular cortex of lymph nodes and subsequently move into the marginal zone where they come in contact with B cells of multiple specificities. Ag-specific B cells located in this region which have bound Ag by IgM alone or in conjunction with CD21 (second and third rows) can be activated and induced to up-regulate costimulatory molecules. These cells can participate in cognate B-T interactions with memory, and perhaps, naive T cells. However, B cells specific for the Ag of interest would be rare at this stage of the immune response, whereas all of the B cells would express CD21 and be capable of binding complement-coated Ag. The participation of these nonspecific CD21-expressing B cells in stimulating Ag-specific primed T cells would increase the likelihood that the immune response would be propagated.

The absence of CD86 expression on B cells participating in CD21-mediated Ag presentation may be important in maintaining tolerance to self-Ag. Naive self-Ag-specific T cells that make their way into the periphery, having escaped central deletion in the thymus, would encounter soluble self-Ag on the surface of CD21-positive B cells. Because CD21 ligation alone in the absence of B cell receptor cross-linking does not induce CD86 expression, the T cells would receive only a single signal through their TCR and would be tolerated. It has been suggested that resting B cells are important in the maintenance of T cell tolerance (35–37), and that CD21 in particular may play a role in this process (38). Interestingly, expression of CD21 is markedly reduced on B lymphocytes of patients with systemic lupus erythematosus (SLE) (39, 40). There is also an early and progressive decrease in this receptor in the MRL/lpr murine model for SLE (41). This decrease is detectable before clinical manifestations of nephritis occur, suggesting that it may play a role in the breakdown of tolerance thought to be important in the pathogenesis of this autoimmune disease.

Although our experiments do not support a role for CD21 in regulating CD86 expression, others have demonstrated that CD21 ligation results in up-regulation of both CD80 and CD86. One study analyzed the effects of incubating complement-containing KLH immune complexes with human PBMC and found that expression of CD80 was induced within 1 h (23). This effect was thought to be due primarily to FcγRII ligation by the immune complexes, with CD21 ligation playing a synergistic role. A second investigation analyzed the responses of resting murine splenic B cells to cross-linking of CD21 with mAbs. In these experiments, the expression of both CD80 and CD86 was up-regulated within 14 h (42). There are several potential explanations for the discrepancy between these results and our own. The first study used unfractionated B cells, and it is possible that the B cell effect was indirect, being triggered by another cell population that actually bound the immune complexes. The results in the second study may result from the use of a different population of B lymphocytes or an alternative method of ligating CD21 or from species specific variations in B cell responses.

The studies described in this report support the role of resting B cells in Ag presentation by means of their ability to bind complement-coated Ag to surface complement receptors. The inability of
B cells to become fully activated in response to CD21 ligation alone may provide the mechanism by which specific Ab responses are maintained, despite the provision of T cell help. Furthermore, the inability of the B cell to up-regulate CD86 may allow the cell to stimulate previously activated T cells specific for foreign Ags, while tolerizing naive T cells specific for self Ags.

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