Lack of induced co-stimulation as a result of complement receptor 2 (CR2) ligation on mouse splenic B cells

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Abstract

B cells act as efficient antigen-presenting cells if they acquire antigen via membrane-bound Ig [termed the B cell receptor (BCR)]. Ligation of the BCR leads to antigen internalization, processing and presentation to CD4⁺ T cells in association with MHC class II molecules. Ligation of the BCR also leads to the generation of activation signals. One short-term consequence of this is the up-regulation of co-stimulatory molecule expression by the B cell, allowing full T cell activation. Other antigen receptors expressed by B cells can also mediate efficient antigen presentation to CD4⁺ T cells. Ligating one such receptor, complement receptor 2 (CR2), has also been described to induce co-stimulatory molecule expression. If correct, this may have serious consequences for ensuring the specificity of the resultant B cell response. We have therefore investigated the effects of ligating both the BCR and CR2 independently of each other, as well as with reagents to cross-link the two receptors, in order to clarify these findings. In contrast to the effects seen upon BCR ligation, we find no evidence for co-stimulatory molecule up-regulation following CR2 ligation. As antigen presentation in the absence of co-stimulation may lead to the induction of tolerogenic or regulatory signals being delivered to T cell populations, these findings imply that the role of CR2 in B cell-mediated antigen presentation is different from that of the BCR.

Introduction

Antibody responses are initiated by individual B cell clones binding and internalizing antigens via their surface Ig B cell receptors (BCR) (1–4). Once delivered into the B cell endocytic environment, the BCR–antigen complex becomes a substrate for proteolysis (5, 6), [reviewed in (7)] in a process similar to that seen in other antigen-presenting cells (APC). This culminates in the generation of small peptide fragments that associate with MHC class II molecules (6, 8, 9). These peptide–MHC (pMHC) complexes are subsequently exported to the cell surface (10, 11) for recognition by CD4⁺ T cells.

Importantly, as well as the pMHC complex, activation of CD4⁺ T cells also requires additional signals from APC, termed co-stimulation. Indeed, if these secondary signals are absent, T cell anergy/death, rather than activation, may result (12).

Many studies have shown that APC express a number of surface co-stimulatory molecules that are members of the B7 molecular family. These provide this second signal by interacting with the constitutively expressed T cell co-stimulation receptor, CD28 [reviewed in (13)]. Accordingly, B cell activation through the BCR has been shown to up-regulate surface expression of both B7-1 (CD80) and B7-2 (CD86) (14, 15). In addition to these two initiating signals (pMHC and co-stimulation), it is also believed that the type of APC affects the developing T cell response. In particular, antigen presentation by B cells has been demonstrated to bias the response toward the T₄₂ phenotype via the ligation of CD40 on the B cell surface with the inducible CD40L on the T cell (15–17), leading to increased T cell IL-4 production. Thus, by a combination of

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BCR-mediated antigen presentation, induced co-stimulation and CD40 expression, B cells can be pivotal in inducing T\(_{h2}\) polarization, eliciting help for their subsequent proliferation and differentiation into antibody-secreting plasma cells.

Although the molecular interactions of B and T cell collaborations following BCR-mediated antigen uptake is becoming clear, substantial evidence exists that B cells can also present antigens that have been captured and internalized by receptors other than the BCR (18–25). In particular, it has been shown in vivo that targeting antigen to the complement receptor 2 (CR2) complex dramatically increases the resulting antibody response (26, 27). This effect has also been studied in vitro, and has been shown to partly be due to the ability of complement ‘tagged’ antigens to cross-link CR2 to the BCR on the surface of B cells (25, 28–32). As well as having a role as a BCR co-receptor, CR2 has also been shown to be capable of mediating antigen presentation independently of the BCR (18, 25, 33, 34). These findings have prompted investigations into the consequences of CR2-mediated antigen presentation in B cells, in particular with respect to the effect on subsequent CD4\(^+\) T cell activation. The interpretation of these experiments will prove crucial in understanding any role of CR2-mediated antigen presentation by B cells. However, the conclusions drawn from these studies are inconsistent. Initial experiments with human (h) B cells demonstrated that, while hCR2-mediated antigen presentation led to T cell stimulation, CD80 expression was not induced unless cross-linking to Fc\(\gamma\)R II was included (33). A later study showed that hCR2-mediated antigen presentation did not lead to the delivery of T cell help and the B cells failed to secrete Ig (35). In contrast, Kozono et al. (36) demonstrated that mouse (m) splenic B cells up-regulated the expression of both CD80 and CD86 following mCR2 ligation. More recently, additional studies (37, 38) have demonstrated that cross-linking hCR2 to the BCR, in the presence of IL-4, enhances up-regulation of both CD80 and CD86 expression compared with BCR ligation alone. However, in the absence of IL-4, this hCR2-mediated enhancement was only witnessed when hCR2/BCR ligation was performed at very low ligand doses.

In this study we have re-examined levels of co-stimulatory molecules expressed on mouse splenic B cells following ligation of either mCR2 or BCR independently, or after cross-linking the two antigen receptors. We clearly demonstrate that, unlike BCR ligation, independent ligation of mCR2 does not lead to any changes in CD80, CD86 or B7 expression levels, either in the presence or absence of IL-4. Furthermore, we show that cross-linking mCR2 to the BCR does not augment levels of CD80, CD86 or B7 above levels induced upon BCR ligation alone. This is also seen when BCR ligation is performed at levels below receptor saturation. The implications of these findings for CD4\(^+\) T cell activation, in the context of previous studies, are discussed.

**Methods**

**B cell isolation**

Using CD43-negative depletion, splenic B cells were isolated from male C57BL/6, BALB/c or mutant mice lacking expression of Fc\(\gamma\)R II (39) (on a BALB/c background (more than eight generations); a kind gift from K. Smith, maintained under the guidelines of the University of Edinburgh's ethical review committee) aged between 6 and 8 weeks. Following incubation with anti-CD43 microbeads (Miltenyi Biotec, Cologne, Germany), the cells were passed through a MACS CS column (Miltenyi Biotec), as per the manufacturer’s instructions. By analyzing B220 (BD Biosciences Pharmingen, San Jose, CA, USA), CD19 [1D3 (40)] and Thy.1 [T24 (41)] expression, preparations used were at least 94% pure for B cells. The eluted CD43-negative cells were then separated on the basis of buoyant density by application to a discontinuous Percoll gradient (Amersham Biosciences, Amersham, UK), spanning densities of 1.06–1.109 g l\(^{-1}\). The activation status of cells recovered from the different bands was monitored by examining expression levels of a number of surface markers (anti-CD80-PE, anti-CD86-PE, anti-CD44-PE (all BD Biosciences Pharmingen) and anti-MHC class II-FITC [M5/114 (42)]) by FACS analysis.

**Antibody \(^{125}\)I-labeling and -binding analysis**

The anti-mCR2 antibody 7E9 mAb [purified from the culture supernatant of cells supplied by T. Kinoshita (43)] was \(^{125}\)I-labeled with Bolton Hunter reagent (Amerham Biosciences) as described (44). To assess the kinetics of its binding, ~2 \(\times 10^6\) CD43-negative B cells were incubated with graded amounts of labeled antibody at 4°C for 1 h in RPMI-1640 supplemented with 2% FCS (First Link, Birmingham, UK). After extensive washing, cell-associated label was measured using a Packard MINAXI gamma counter. Specificity of binding was checked by the inclusion of samples containing 100-fold excess non-labeled 7E9, typically quenching cell-associated label by 95%.

**B cell treatments**

mAbs to either mBCR or mCR2 were used as a means of receptor ligation. To target the BCR, biotin-conjugated F\((\text{ab'})_2\) fragment, goat anti-mouse IgM (Jackson Immunoresearch Laboratories, West Grove, PA, USA) antibodies were used at a concentration of 3.4 \(\mu\)g ml\(^{-1}\), unless stated otherwise. mCR2 was targeted with either 2 \(\mu\)g ml\(^{-1}\) of the biotinylated mAbs 7E9 or 7G6 (both of which also bind the closely related molecule, CR1) (43) or with a recombinant antigen consisting of three tandem copies of the natural ligand for mCR2 (mC3d) fused to the C-fragment of the bacterial antigen tetanus toxin (TTCF) [(C3d)\(_3\)–TTCF] (25, 45). To target Fc\(\gamma\)R II, biotinylated anti-Fc\(\gamma\)R II/III/mAb 2.4G2 (46) was used (2 \(\mu\)g ml\(^{-1}\)). To simulate extensive cross-linking of the individual receptors, 5 \(\mu\)g ml\(^{-1}\) streptavidin (Southern Biotechnology Associates, Birmingham, AL, USA) was also added. To cross-link the various antigen receptors (CR2 to BCR, CR2 to Fc\(\gamma\)R II) biotinylated anti-CR2, -BCR or -Fc\(\gamma\)R mAbs were added simultaneously in the appropriate combinations followed by 5 \(\mu\)g ml\(^{-1}\) streptavidin. Cells were cultured at 2 \(\times 10^6\) ml\(^{-1}\) in either 24- or 48-well plates (Corning B.V. Life Sciences, Schiphol-Rijk, The Netherlands) at 37°C, in an atmosphere of 5% CO\(_2\), in RPMI-1640 supplemented with 10% FCS, 100 \(\mu\)g ml\(^{-1}\) kanamycin, 2 mM glutamine, 1 mM sodium pyruvate, non-essential amino acids (all Invitrogen Ltd, Paisley, UK) and 50 \(\mu\)M \(\beta\)-mercaptoethanol (Sigma, Poole, UK) (complete.
Where indicated, media were supplemented with 10 ng ml\(^{-1}\) mIL-4.

**Proliferation assays**

To measure antibody-induced proliferation, ~1.5 × 10\(^5\) cells were incubated, in duplicate, in 96-well plates with the various antibody treatments (at concentrations described above) either in the presence or in the absence of mIL-4 for 65 h with 18.5 kBq \(^{[3]}\)H\)thymidine \((^{[3]}\)H\)TdR\) (Amersham Biosciences). \(^{[3]}\)H\)TdR incorporation, measured by scintillation counting, was taken as a direct measurement of cell proliferation.

**FACS analysis**

Cells were incubated with directly labeled antibodies for 30 min at 4°C in PBS + 2% FCS before thorough washing. A total of 10 000 events were collected on a Becton Dickinson FACSCalibur and analyzed using FlowJo 4.6.2 (Tree Star Inc., Ashland, OR, USA). FcR\(\gamma\)II binding of all antibodies was controlled for using the blocking anti-FcR\(\gamma\)II mAb 2.4G2 (BD Biosciences Pharmingen).

**Results**

**Isolation, analysis and activation of mouse B cells**

In order to assess the consequences of mCR2-mediated antigen presentation by B cells, with respect to their ability to stimulate T cells, we decided to clarify the levels of co-stimulatory molecules CD80 and CD86 on mouse splenic B cells following mCR2 ligation. To ensure that the levels of co-stimulatory molecules were not affected by the purification procedure itself, we isolated splenic B cells by negative selection using CD43 magnetic beads. CD43 is a transmembrane molecule expressed by a number of lymphoid cell types, but importantly, is absent on the majority of B cells found in the secondary lymphoid organs (termed B-2 B cells) (47). Approximately 94% of the resulting CD43-negative population were shown to be positive for the B cell markers B220 and CD19 (data not shown). These cells were then subjected to discontinuous Percoll-gradient centrifugation to allow the removal of any activated cells. Figure 1(A) shows the expression profiles of a panel of activation markers on the B cell fractions following centrifugation. It can be seen that the B cells pooled from bands 1, 2 and 3 (termed high density) expressed considerably lower levels of the co-stimulatory markers CD80 and CD86 (as well as the other markers analyzed) than the cells pooled from the other bands. Cells pooled from these lower three bands were therefore used throughout this study.

**Anti-mCR2 mAbs do not induce splenic B cell proliferation or up-regulation of co-stimulatory molecule expression**

As previous reports had demonstrated that anti-CR2 mAbs failed to induce human B cell proliferation (48), but did induce up-regulation of both CD80 and CD86 on mouse splenic B cells (36), we sought to address this apparent discrepancy. We therefore repeated both of these experiments with negatively isolated, naive mouse splenic B cells using the 7E9 anti-mCR2 mAb. This mAb was confirmed to bind splenic B cells with saturable kinetics (~1–2 \(\mu\)g ml\(^{-1}\)) (Fig. 1B), and was calculated to react with ~20 000 binding sites/CD43-negative cell, comparable to that seen in other studies (25, 49).

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**Fig. 1.** Analysis of cell-surface markers expressed on CD43-negative B cells. (A) Levels of activation markers were assessed on cells after discontinuous Percoll-gradient centrifugation. Cells from bands 1, 2 and 3 were pooled (shaded), as were cells from bands 4 and 5 (hatched), and analyzed for the expression of CD80, CD86, MHC class II (MHC II) and CD44 by FACS. Results are presented as mean fluorescence intensity (MFI) in arbitrary units. (B) Levels of anti-mCR2 mAb (7E9) binding to total CD43-negative B cells. Asterisk (*) represents the level of binding of 2 \(\mu\)g ml\(^{-1}\) \(^{[125]}\)I 7E9 mAb in the presence of 200 \(\mu\)g unlabeled mAb (~3.5% of the binding seen in the absence of unlabeled mAb).
We therefore performed the following experiments using the 7E9 anti-mCR2 mAb to ligate mCR2 on the high-density, CD43-negative B cells. As expected, treatment of B cells with biotinylated anti-BCR mAb (in the presence of cross-linking streptavidin) induced proliferation as measured by [3H]TdR incorporation over a 65-h period. This can be increased by the inclusion of IL-4 in the culture media (Fig. 2A). In stark contrast, anti-mCR2 treatment (also in the presence of cross-linking streptavidin), either in the presence or in the absence of IL-4, failed to induce any proliferation above that seen in media controls.

We then examined the levels of CD80 and CD86 expressed by B cells after BCR ligation for a shorter period of 17 h. Figure 2(B) shows, in agreement with a previous report (14), that, while there is no difference in CD80 expression during this time period, there is an increase in CD86 expression after BCR ligation. This increase in CD86 expression can also be enhanced through the addition of IL-4 (Fig. 2C). However, when the B cells were treated with the anti-mCR2 mAbs, there was no observed increase in CD80 or CD86 expression in either the absence (Fig. 2B) or presence (Fig. 2C) of IL-4. Thus, mCR2 ligation does not induce co-stimulatory molecules on the surface of mouse splenic B cells after 17 h. In addition, the cross-linking of mCR2 to the BCR failed to induce any further CD86 up-regulation compared with that seen with the anti-BCR treatment only (Fig. 2B and C, bottom panels).

We next decided to address whether mCR2 ligation may have an effect on CD86 expression after a longer period of

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Fig. 2. Anti-mCR2 antibody, 7E9, fails to induce either proliferation or altered expression of the co-stimulatory molecules, CD80 or CD86, on purified splenic B cells. CD43-negative, high-density splenic B cells were incubated with media-alone, biotinylated mAbs directed against BCR (3.4 μg ml⁻¹) or mCR2 (7E9; 2 μg ml⁻¹) or a combination of both anti-BCR and mCR2 mAbs. Streptavidin (5 μg ml⁻¹) was added to the wells containing mAbs to cross-link the ligated receptors. In (A), proliferation was assessed by incorporation of [3H]TdR in the absence (empty bars) or presence (filled bars) of 10 ng ml⁻¹ IL-4. Alternatively, cells were incubated for 17 h either in the absence (B) or in the presence (C) of 10 ng ml⁻¹ IL-4 before the levels of CD80 and CD86 were analyzed.
time. Figure 3(A) shows that, even after 4 days in the presence of IL-4, mCR2 ligation failed to induce any increase in CD86 expression. As it was apparent that anti-BCR-induced CD86 expression actually declined after 4 days, we decided to explore the possibility that we had yet to find the optimal time period for any anti-mCR2-induced effects. Figure 3(B) shows a time course for CD86 expression levels after various antigen receptor ligation treatments in the presence of IL-4. Anti-BCR ligation induces a rapid up-regulation of CD86, which peaks after 24 h and then declines over the next 3 days. In contrast there is no evidence for CR2-induced CD86 up-regulation over this period.

Recent studies have demonstrated that other members of the B7 family of co-stimulatory molecules also play an important role in APC-mediated T cell stimulation [reviewed in (50)]. In particular, it has been shown that both B7h (51) and 4-1BBL (52) are expressed by B cells. These co-stimulatory molecules appear to be involved in later T cell activation events by binding the inducible co-stimulator molecules expressed on activated T cells, ICOS and 4-1BB. B7h expression has also been shown to be required for CD4+ T cell polarization (53) and is modified by BCR ligation (54). We therefore analyzed both B7h and 4-1BBL following various antigen receptor ligation. As can be seen in Fig. 3(C),
anti-BCR ligation led to reduced B7h expression [as previously reported (54)] after 17 h, and did not affect 4-1BBL levels. In contrast, anti-mCR2 treatment had no effect on B7h or 4-1BBL after 17 h (Fig. 3C) or indeed after 4 days (data not shown).

Previously, Kozono et al. (36) had demonstrated that distinct anti-mCR2 mAbs behave differently in their ability to up-regulate CD80 and CD86. 7E9 binds to an epitope other than that involved in natural ligand (C3d) binding to mCR2, whereas another anti-mCR2 mAb 7G6 directly competes for C3d-binding sites (55). Although Kozono et al. demonstrated that 7E9 was more efficient than 7G6 at inducing CD80 and CD86 expression, we wanted to see if alternative methods of cross-linking mCR2 would induce co-stimulatory molecule expression in our experiments. We therefore repeated these experiments using either this alternate anti-mCR2 mAb (7G6) or a recombinant form of the natural ligand for mCR2 (C3d)3 fused to the bacterial antigen, the TTCF (45) that is efficiently presented to TTCF-specific T cells in a mCR2-dependent manner (25). As can be seen in Fig. 4, anti-mCR2 mAb 7G6, like 7E9, fails to increase expression of CD80 and CD86, both

![Fig. 4](image-url)

**Fig. 4.** Alternate mCR2 ligation also fails to alter levels of co-stimulatory molecules CD80 and CD86 on purified splenic B cells. In (A) and (B), CD43-negative, high-density splenic B cells were incubated with media-alone, biotinylated mAbs directed against BCR (3.4 μg ml⁻¹) or mCR2 (7G6; 2 μg ml⁻¹) or a combination of both anti-BCR and mCR2 mAbs. Streptavidin (5 μg ml⁻¹) was added to the wells containing mAbs to cross-link the ligated receptors. Cells were incubated for 17 h either in the absence (A) or in the presence (B) of 10 ng ml⁻¹ IL-4 before the levels of CD80 (left-hand side histograms) and CD86 (right-hand side histograms) were analyzed. In (C), cells were incubated in media containing IL-4 alone, in media containing IL-4 and biotinylated mAbs directed against mCR2 (7E9; 2 μg ml⁻¹) or in media containing IL-4 and (C3d)₃-TTCF (2 μg ml⁻¹). After 17 h the levels of CD80 (left-hand side histograms) and CD86 (right-hand side histograms) were analyzed.
in the absence (Fig. 4A) and presence (Fig. 4B) of IL-4, after 17 h or 4 days (data not shown). Likewise, the addition of the natural mCR2 ligand-containing (C3d)3–TTCF in the presence of IL-4 also fails to induce any CD80 or CD86 increased expression after incubation for either 17 h (Fig. 4C) or 4 days (not shown).

Lack of anti-mCR2 mAb-induced CD86 expression is not due to FcR engagement

Although the reagents used in this study appear not to bind to FcRγII expressed by B cells (by blocking studies; not shown), we decided it was important to rule out any possibility that our intact antibodies (used as surrogate receptor ligands) were inducing FcRγII cross-linking. This is of particular importance since many stimuli delivered through the BCR, for example, are negated after cross-linking to FcRγIIIB (56). As it has been documented that mice lacking this FcR do not have any B cell lineage disruption, but do fail to regulate BCR-mediated B cell stimuli, we repeated our antibody treatments using B cells purified in an identical manner from mice lacking FcRγII expression. As these mice are bred on a BALB/c background, we also performed control wild-type (WT) BALB/c B cell isolations in parallel. It can be seen in Fig. 5 (similar to that seen with B cells isolated from C57BL/6 mice; Figs 2–4) that incubation of B cells from either WT or FcRγII null mice (FcRγII KO) with mAbs to mCR2 does not induce the up-regulation of either CD80 or CD86 expression. In contrast, when biotinylated mAbs to both the BCR and mCR2 are added to high-density B cells [from both types of mice (Figs 4 and 5)], CD86 expression is seen to increase. Thus, the failure of mCR2 to alter levels of CD80 or CD86 (as well as anti-BCR-mediated CD86 up-regulation) appears independent of FcRγII ligation.

mCR2 cross-linked to the BCR at low ligand occupancy fails to up-regulate CD86 co-stimulatory activity

In previous studies it has been reported that, in the absence of IL-4, BCR-mediated up-regulation of CD80 and CD86 may be enhanced by hCR2 cross-linking, but only under conditions of very low BCR ligand occupancy (38). As we had failed to detect any additive effect of mCR2 cross-linking to the BCR (Figs 2–4) with regard to CD80/86 expression levels with standard saturating levels of anti-BCR mAb (3.4 μg ml−1), we next decided to examine CD86 levels after cross-linking the anti-mCR2 mAb, 7E9, to limiting doses of anti-BCR mAb. Figure 6(A) shows that, in the presence of IL-4, lowering the level of BCR occupancy appears to have comparatively little effect on levels of CD86 (open bars), due to the relative high levels induced by the presence of IL-4 alone (hatched bar; media). In addition, when the anti-mCR2 mAb is included (filled bars), the levels of CD86 expression appear very similar. In the absence of IL-4 (Fig. 6B), as expected, reducing the anti-BCR mAb (open bars) to levels as low as 0.03 μg ml−1 reduces the level of CD86 expression to that seen in the

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**Fig. 5.** Cross-linking mCR2 on B cells lacking FcRγII fails to alter CD86 expression levels. CD43-negative, high-density splenic B cells purified from either WT or FcRγII null mice (39) were incubated with media-alone, 2 μg ml−1 biotinylated anti-mCR2 (7E9) mAbs or a combination of biotinylated anti-CCR2 (7E9; 2 μg ml−1) with biotinylated anti-BCR (3.4 μg ml−1) or with biotinylated anti-FcRγII (2.4G2; 2 μg ml−1) mAbs. Streptavidin (5 μg ml−1) was added to the wells containing mAbs to cross-link the ligated receptors. Cells were then incubated for 17 h in the presence of 10 ng ml−1 IL-4 before the levels of CD80 and CD86 were analyzed.
mCR2 does not induce co-stimulation on B cells

Discussion

Previously, in vitro studies from our laboratory (25), as well as those from others (18, 33, 34), have shown that both human and mouse CR2, expressed on B cells, are capable of mediating efficient antigen presentation for CD4+ T cell recognition. Our findings, that distinct sequences within the cytoplasmic domain of mCR2 are essential for this, as well as for enhancing BCR-mediated presentation (25), raise the possibility that CR2-mediated antigen presentation may occur in vivo. Unlike BCR-mediated antigen presentation, however, hCR2-mediated antigen presentation leads to insufficient T cell stimulation for subsequent B cell differentiation and antibody secretion (35). To try to understand the consequences of CR2-mediated antigen presentation, several studies have looked at the levels of co-stimulatory molecule expression following engagement of either the BCR or CR2 on various populations of B cells. It was shown with human B cells that CR2 ligation alone failed to up-regulate CD80 (33), although CD86 expression was not examined in this report. It was therefore surprising that Kozono et al. (36) demonstrated that mCR2 ligation appeared to induce similar levels of CD80 and CD86 to those seen upon BCR ligation. In our previous studies investigating the role of the mCR2 cytoplasmic domain in antigen presentation, CH27 B cells transfected with mCR2 were used (25). Unfortunately, as these cells constitutively express high levels of co-stimulatory molecules, we were unable to detect any differences in their expression after the ligation of WT or various cytoplasmic domain mutants of mCR2 (D. V. Barrault and A. M. Knight, unpublished results).

In this report, we have examined the levels of CD80 and CD86 following antigen receptor ligation in freshly isolated mouse B cells. Unlike Kozono et al. (36), but consistent with Thornton et al. (33) using human B cells, we find no evidence of CR2-induced co-stimulatory molecule expression. In the study of Kozono et al., mCR2 was ligated using anti-CR2 mAbs followed by cross-linking with intact anti-rat secondary antibodies. While these secondary reagents were shown to lack anti-BCR cross-reactivity, cross-linking to the B cell FcγRII was not excluded (36). Indeed, in the study of Thornton et al. (33), when CR2 was cross-linked to FcγRII, CD80 levels were seen to be elevated. In our present study, the anti-CR2 monoclonals were checked prior to use and shown to be negative for FcγRII binding. The cross-linking in our study was achieved using streptavidin rather than secondary antibodies, so removing any possible FcR engagement. More importantly, we have also generated identical results, after ligating mCR2 on B cells purified from mice lacking expression of FcγRII.

Somewhat surprisingly, however, when we specifically cross-linked mCR2 to FcγRII on B cells using the 2.4G2 mAb, no increased levels of CD80 or CD86 were seen. It may be that while the binding of the 2.4G2 mAb by FcγRII does block subsequent antibody, its binding does not replicate the binding seen with Fc regions of intact antibodies as were used by Kozono et al. (36). Alternately, the results from the study of Kozono et al. may be due to other differences in the experimental systems. However, our present study, in agreement with others previously reported, clearly demonstrates that, while capable of efficient antigen presentation independent of other antigen receptor (e.g. BCR, FcγRII) engagements (18, 25, 33, 34), mCR2 performs this in the absence of increased co-stimulatory molecule expression.

These findings demand an explanation for the role of CR2-mediated antigen presentation by B cells. In the absence of any co-stimulation expressed by APC, responding T cells are stimulated sub-optimally and may develop into an 'anergic'
phenotype. This can lead to either permanent or transient unresponsiveness, cell death or the development of regulatory function [reviewed in (13)]. Thus, only the very limited number of B cell clones capable of capturing and presenting antigen via specific BCR expression supply the correct co-stimulatory signals to responding CD4^+ T cells.

So what is the role of CR2-mediated antigen presentation leading to sub-optimal signal delivery? Clearly, one advantage is that the numbers of B cells that can act as non-specific APC are vast, compared with those expressing an antigen-specific BCR. Maybe, by accumulating large numbers of sub-optimal co-stimulatory signals, received from B cells presenting antigen via CR2, T cells may use this abundant population as APC. Clearly though, this sub-optimal signal is not sufficient to induce cognate B cell help, and indeed, irrelevant antibody secretion does not occur. It may, however, result in an increase in antigen-specific CD4^+ T cells providing non-cognate or ‘bystander help’ to enhance the cognate ‘help’ received by antigen-specific B cells. Additional studies will have to be performed to test this hypothesis.

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Abbreviations

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<th>Term</th>
<th>Description</th>
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<td>APC</td>
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References


