

Intersection of the Complement and Immune Systems: A Signal Transduction Complex of the B Lymphocyte-containing Complement Receptor Type 2 and CD19

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Summary

The complement system augments the humoral immune response, possibly by a mechanism that involves the B lymphocyte membrane receptor, CR2, which binds the C3dg fragment of C3 and triggers several B cell responses in vitro. The present study demonstrates that CR2 associates with a complex of membrane proteins that may mediate signal transduction by ligated CR2. Monoclonal antibodies to CR2 immunoprecipitated from digitonin lysates of Raji B lymphoblastoid cells a membrane complex containing CR2, approximately equimolar amounts of CD19, which is a member of the immunoglobulin superfamily, and three unidentified components: p130, p50, and p20. The complex, which was immunoprecipitated also with anti-CD19, could be dissociated by Nonidet P-40, accounting for its absence in previous studies of CR2. Expression of recombinant CR2 and CD19 in K562 erythroleukemia cells led to formation of a complex that contained not only these two proteins but also p130, p50, and p20, and another component, p14. These unidentified components of the CR2/CD19 complex coimmunoprecipitated with CD19 and not with CR2 from singly transfected cells, indicating primary association with the former. CD19 replicated the capacity of CR2 to interact synergistically with mIgM for increasing free intracellular Ca^{2+} , suggesting that the complex mediates this function of CR2. Therefore, CR2 associates directly with CD19 to become a ligand-binding subunit of a pre-existing signal transduction complex of the B cell that may be representative of a family of membrane protein complexes. This interaction between the complement and immune systems differs from that between immunoglobulin and Clq by involving membrane rather than plasma proteins, and by having complement involved in the afferent phase of the immune response.

The B lymphocyte has membrane receptors by which it responds to immunologic and nonimmunologic stimuli; the former are members of the Ig superfamily, and the latter are receptors of the complement system. Of the former, membrane Ig (mIg)¹ mediates clonally restricted uptake of antigen by the B cell, and MHC class II directs antigen presentation to T lymphocytes. In addition, mIg activates phospholipase C (PLC) (1) by a pathway that involves guanine nucleotide binding proteins (2) and protein tyrosine kinases (3), and is a part of a multimolecular complex (4–6), the other components being required at least for membrane

expression of mIg and possibly for signal transduction. The Fc_γ receptor type II ($Fc_\gamma RII$), another member of the Ig superfamily, modulates this pathway by suppressing PLC activation by crosslinked mIg (7). MHC class II, which would be ligated during cognate B-T cell interactions, also induces translocation of protein kinase C to the nucleus (8), and proliferation and differentiation of primed B cells (9).

These reactions of the B cell, which are fundamental to the humoral immune response, do not require components of the complement system, all of which are evolutionarily distinct from the Ig superfamily. However, genetic or acquired deficiencies of certain components of the classical pathway are associated with diminished primary antibody responses to T-dependent and -independent antigens (10–13), impaired generation of memory B cells (14), low levels of certain Ig

¹ Abbreviations used in this paper: CR, complement receptor; DTT, dithiothreitol, mIg, membrane Ig; PLC, phospholipase C; SCR, short consensus repeat.

isotypes (15), and the occurrence of autoimmune diseases characterized by the presence of autoantibodies (16). Only deficiencies of complement proteins that are involved in the activation of C3 by the classical pathway, or of C3 itself, are associated with altered immune responses, and activated C3 serves as the ligand for complement receptors on B cells. Therefore, the complement system, which shares with the immune system a common purpose of host defense against microbial infection, can modulate B cell responses *in vivo*, and the intersection between these two systems is likely to involve C3 receptors on B lymphocytes.

Proteolytic activation of C3 generates the C3b fragment that covalently attaches to the activator of the complement system. The bound C3b transiently serves as a ligand for complement receptor type 1 (CR1; CD35) before it is trimmed down to the C3dg fragment, which has a new specificity for CR2 (CD21). CR1 and CR2 are expressed by all mature B cells (17, 18), are homologous structures encoded by closely linked genes on chromosome 1q32 (19–21), and contain a tandemly repeating, 60–70–amino acid motif, termed the short consensus repeat (SCR) (22–24). The most common forms of CR1 and CR2 have 30 and 15 SCRs, respectively, that constitute their entire extracytoplasmic domains, single membrane spanning segments, and relatively short COOH-terminal cytoplasmic regions of 43 and 34 amino acids, respectively. The tandemly aligned SCRs form filamentous structures (25, 26) that extend the ligand binding sites away from the plasma membrane.

The potential of CR2 for augmenting B cell function has been shown by the capacity of mAbs or polymeric C3dg to induce B cell proliferation in the presence of T cell–derived factors (27) or PMA (28), to prime B cells for mIgM-dependent proliferation (29), and, when crosslinked to mIgM, as might occur with immune complexes containing both C3dg and antigen, synergistically to enhance increases in free intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (30), perhaps by a process related to that mediating modest increases in $[Ca^{2+}]_i$ when CR2 is crosslinked alone (31). Ligation of CR2 also maintains the growth of human B lymphoblastoid cells in suboptimal culture conditions (32, 33), and of murine B cells that had been stimulated with LPS (34). Thus, CR2 meets an essential requirement for a molecule that might link the complement and immune systems, that of modulating B cell function when ligated by a product of complement activation.

The possible association of CR2 with other membrane proteins was assessed because the short cytoplasmic extension of CR2 seemed insufficient to mediate these multiple B cell responses. In digitonin extracts of B lymphoblastoid cells, CR2 was demonstrated to be a component of a multimolecular complex that contains CD19, a member of the Ig superfamily (35, 36) that is expressed throughout the ontogeny of the B cell (37), and three to four other membrane proteins. The additional, unidentified components were also detected in K562 erythroleukemia cells in which the complex was reconstituted by the transfection with CR2 and CD19 cDNA constructs. Therefore, there resides on B cells a signal transduction complex separate from the antigen receptor complex that is comprised of B cell–restricted proteins, CR2 and

CD19 of the complement and immune systems, respectively, and other components that are also present in non-B cells, where they may function in homologous signal transduction complexes containing other ligand-binding components.

Materials and Methods

mAbs. The anti-CR2 mAbs HB5 (American Type Culture Collection [ATCC], Rockville, MD) and OKB7 (Ortho Diagnostic Systems Inc., Westwood, MA), the anti-CD19 mAbs B4 (37), 4G7 (Becton Dickinson, & Co., Mountain View, CA), and HD37 (Dako Corp., Santa Barbara, CA), and DA4.4 anti-IgM (ATCC) were used. Control antibodies for nonspecific immunoprecipitation were RPC5.4 IgG2a (ATCC) and MOPC21 IgG1 (Cappel Laboratories, Malvern, PA).

Preparation of K562 Erythroleukemia Cells Expressing CR2 and CD19. Replicate samples of 10^6 K562 cells, which had been grown in RPMI supplemented with 10% FCS, penicillin, and streptomycin, were pelleted and resuspended with 1.5 ml of RPMI containing 0.03 ml Lipofectin (Bethesda Research Laboratories, Bethesda, MD) and either 38 μ g of pICR2 (38) and 2 μ g pSVneo.1 for expression of CR2, or 40 μ g of pZipneo.SV(CD19) for expression of CD19. After culture for 24 h, 3 ml of RPMI with 20% FCS was added and culture continued for 24 h. The cells were washed, cultured for an additional 24 h in RPMI/10% FCS, and then selected by growth in medium containing 400 μ g/ml G418 (Gibco Laboratories, Grand Island, NY). The G418-resistant cells were immunofluorescently stained with HB5 anti-CR2 or HD37 anti-CD19 and sorted for high CR2- or CD19-expressing cells by use of a FACStar-Plus flow cytometer (Becton Dickinson & Co.).

K562 cells expressing both CR2 and CD19 were prepared by cotransfection of CR2-expressing cells with pZipneo.SV(CD19) and p141, a plasmid conferring resistance to hygromycin, followed by selection in medium containing 200 μ g/ml hygromycin B (Calbiochem-Behring Corp., San Diego, CA). Cells expressing high CD19 and CR2 were sorted by use of the FACStar-Plus.

Immunoprecipitation of Radiolabeled Cells. Raji B lymphoblastoid cells and K562 transfectants were surface labeled with ^{125}I (New England Nuclear, Boston, MA) by a procedure that involves lysine residues (39). Raji cells were labeled *in vivo* with $^{32}PO_4$ (New England Nuclear), and replicate samples were incubated for 10 min at 20°C in buffer alone or containing 100 ng/ml PMA (40). Raji cells were also labeled by culture for 16 h in methionine-depleted medium to which had been added 0.1 mCi [^{35}S]methionine (41). Cells were lysed in buffer containing 1% digitonin (Sigma Chemical Co., St. Louis, MO) (42), 10 mM triethanolamine, pH 7.4, 150 mM NaCl, 1 mM EDTA, 5 mM DFP, 1 μ g/ml antipain, 1 μ g/ml chymostatin, 1 μ g/ml pepstatin, and 1 μ g/ml leupeptin. In the phosphorylation experiments, the digitonin buffer also contained 10 mM NaF, 5 mM $Na_4P_2O_7$, 1 mM Na_3VO_4 , 1 mM ATP, and 100 μ g/ml DNase (40). For lysis with 1% NP-40, this detergent was substituted for digitonin.

For immunoprecipitation of CR2, insoluble material was removed by centrifugation of the lysates from 2×10^7 cells/0.6 ml lysis buffer. The lysates were precleared twice by rocking at 4°C for 4 and 24 h, with 50 μ l packed Trisacryl beads bearing protein A (Pierce Chemical Co., Rockford, IL), and were divided into replicate samples to which were added 10 μ g HB5 and 10 μ g RPC5.4 IgG2a, respectively, and 25 μ l protein A-Trisacryl beads. After rocking for 1 h at 4°C, the beads were pelleted, washed, and eluted into 0.1 ml of boiling sample buffer containing 6% SDS and 200 mM dithiothreitol (DTT). For immunoprecipitation of CD19, 5

μg HD37 or B4 anti-CD19, and MOPC21 IgG1 were substituted for HB5 and RPC5.4, respectively, and the complexes were recovered with rabbit Ig anti-mouse Ig coupled to Sepharose (Dako Corp.). The eluates of all immunoprecipitates were analyzed on 5–15% SDS-polyacrylamide gradient gels. Two-dimensional non-reducing/reducing gels were run on 5–20% gradient gels in the first dimension without prior treatment with DTT, the gel lanes were cut out, soaked in sample buffer containing 5% 2-ME for 1 h, and layered on a 5–20% gradient gel with a 3% stacking gel for the second dimension. IEF/SDS gels were performed by the method of O'Farrell (43) with the second dimension run in a 5–20% polyacrylamide gradient.

Analysis of $[\text{Ca}^{2+}]_i$ in Daudi Cells. Daudi B lymphoblastoid cells were loaded with indo-1-AM (Molecular Probes, Eugene, OR), and incubated with mAbs specific for mIgM, CR2, and CD19, respectively. The ratio of fluorescence at 400–415 nm to 485–500 nm was measured for each cell by flow cytometry using the FACStar-Plus and the Chronos Program (Becton Dickinson & Co.). The mean ratio for all cells in consecutive 10-s intervals was determined for 20 s before and 140 s after addition of crosslinking goat F(ab')₂ anti-mouse Ig that had been previously adsorbed with immobilized human IgM. These measurements were converted to mean $[\text{Ca}^{2+}]_i$ (44).

Results

CR2 and CD19 Are Components of a Multimolecular Membrane Protein Complex of B Lymphocytes. Raji B lymphoblastoid cells that had been surface labeled with ¹²⁵I were solubilized in buffer containing digitonin, and immunoprecipitates were prepared with two anti-CR2 mAbs, HB5 and OKB7, and analyzed by SDS-PAGE. In addition to CR2 at 145 kD, four additional labeled proteins designated according to their molecular masses as p130, p95, p40, and p20, respectively, were specifically precipitated with both HB5 and OKB7 (Fig. 1). As HB5 and OKB7 recognize different epitopes on CR2 (38), it is unlikely that the coprecipitating proteins were present by crossreacting with the anti-CR2 mAbs.

The p95 coprecipitating with CR2 resembled the B cell-specific membrane protein, CD19, by molecular mass. Therefore, replicate samples of a digitonin lysate of ¹²⁵I-labeled Raji cells were subjected to immunoprecipitation with monoclonal anti-CR2 and anti-CD19, respectively, followed by SDS-PAGE. The anti-CR2 immunoprecipitated five proteins that resembled those observed in the experiment depicted in Fig.

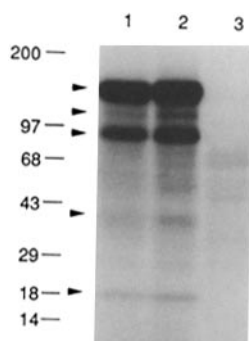


Figure 1. Autoradiograph of SDS-polyacrylamide gel of immunoprecipitates obtained from digitonin lysates of ¹²⁵I surface-labeled Raji B lymphoblastoid cells with HB5 anti-CR2 (lane 1), OKB7 anti-CR2 (lane 2), and control RPC5.4 IgG2a (lane 3), respectively. The arrowheads indicate the positions of proteins specifically immunoprecipitated by both anti-CR2 mAbs.

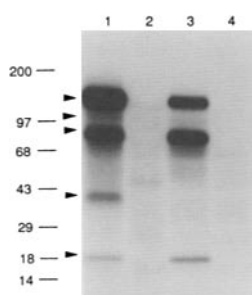


Figure 2. Autoradiograph of SDS-polyacrylamide gel of immunoprecipitates obtained from digitonin lysates of ¹²⁵I-labeled Raji B lymphoblastoid cells with HB5 anti-CR2 (lane 1), control RPC5.4 IgG2a (lane 2), HD37 anti-CD19 (lane 3), and control MOPC21 IgG1 (lane 4), respectively.

1 (Fig. 2, lane 1). The anti-CD19 recovered four of these components, corresponding to CR2 at 145 kD and CD19 at 95 kD, p130 and p20; p40 was not present (Fig. 2, lane 3). The different relative intensities of the CR2 and CD19 bands in the anti-CR2 and anti-CD19 precipitates may be caused by the approximately fivefold larger number of CD19 than of CR2 molecules that are expressed by Raji cells, or may indicate either that anti-CR2 does not recover all CD19 or that anti-CD19 does not recover all CR2.

Prior studies that had demonstrated only CR2 in immunoprecipitates obtained with antibody of this specificity from lysates of B lymphocytes that had been surface labeled with ¹²⁵I (45, 46) had used the nonionic detergents, NP-40 or Triton X100, for solubilizing membrane proteins. The role of digitonin in maintaining the CR2/CD19 complex was assessed by solubilizing replicate samples of ¹²⁵I-labeled Raji cells in digitonin and NP-40, respectively, and analyzing immunoprecipitates obtained either with anti-CR2 or anti-CD19. In the presence of NP-40, anti-CR2 precipitated only three proteins, CR2, p130, and p40 (Fig. 3 A, lane 2), of the five that were seen in the presence of digitonin (Fig. 3 A, lane 1). Similarly, in the presence of NP-40, anti-CD19 precipitated CD19 (Fig. 3 B, lane 2), but not CR2, p130, and p20, which were present in immunoprecipitates prepared from digitonin lysates (Fig. 3 B, lane 1). Thus, the association of CR2, CD19, and p20 is dependent on the use of digitonin.

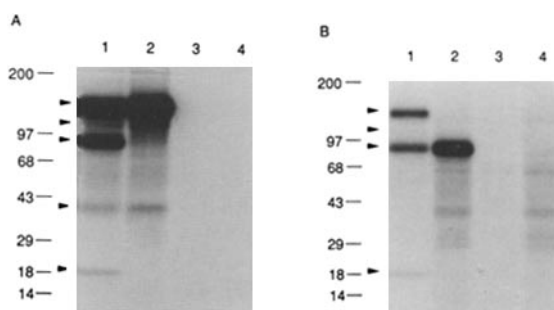


Figure 3. (A) Autoradiograph of SDS-polyacrylamide gel of immunoprecipitates obtained from digitonin lysates (lanes 1 and 3) and NP-40 lysates (lanes 2 and 4) of ¹²⁵I-labeled Raji cells with HB5 anti-CR2. (B) Autoradiograph of SDS-polyacrylamide gel of immunoprecipitates obtained with HD37 anti-CD19 from replicate samples of the digitonin lysates (lanes 1 and 3) and NP-40 lysates (lanes 2 and 4) of ¹²⁵I-labeled Raji cells.

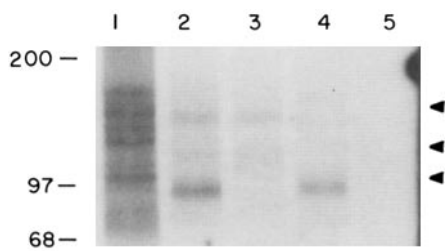


Figure 4. Autoradiograph of SDS-polyacrylamide gel of the membrane protein reimmunoprecipitated by anti-CD19 from the NP-40/SDS eluate of the complex obtained from digitonin lysates of $^{32}\text{PO}_4$ -labeled, PMA-treated Raji cells by anti-CR2. Immunoprecipitates were obtained with control RPC5.4 IgG2a (lane 1) and HB5 anti-CR2 (lane 2), respectively, from replicate samples of the digitonin lysate of in vivo $^{32}\text{PO}_4$ -labeled Raji cells. The latter was eluted by treatment with 2% NP-40/0.2% SDS, and the proteins remaining bound to the HB5-Sepharose were analyzed (lane 3). The eluate was reimmunoprecipitated with HD37 anti-CD19 (lane 4) and control MOPC21 IgG1 (lane 5), respectively.

To determine whether the membrane proteins coprecipitating with CR2 could be phosphorylated in the presence of PMA, and whether the 95-kD protein in the immunoprecipitate could be demonstrated to react with anti-CD19, Raji cells were incubated with $^{32}\text{PO}_4$ and treated with PMA to induce phosphorylation of CR2 (40). Anti-CR2 immunoprecipitated from digitonin lysates of the Raji cells three phosphoproteins corresponding in molecular mass to CR2, p130, and CD19 (Fig. 4, lane 2). Treatment of a replicate sample of the anti-CR2 immunoprecipitate with NP-40/SDS at 4°C released the 95-kD protein from the complex (Fig. 4, lane 3), and this protein was specifically immunoprecipitated from the NP-40/SDS eluate with anti-CD19 (Fig. 4, lane 4), providing direct evidence for its identity as CD19. The 130-kD component of the complex was not released by NP-

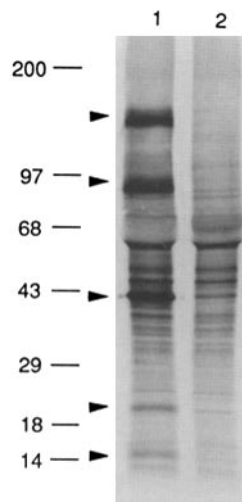


Figure 5. Autoradiograph of SDS-polyacrylamide gel of proteins immunoprecipitated by HB5 anti-CR2 (lane 1) and control RPC5.4 IgG2a (lane 2), respectively, from digitonin lysates of Raji cells biosynthetically labeled with [^{35}S]-methionine.

40/SDS, consistent with its coprecipitation with CR2 in the presence of NP-40 (Fig. 3).

Raji cells were biosynthetically labeled with [^{35}S]-methionine, and immunoprecipitates were prepared with HB5 anti-CR2 from digitonin lysates. In addition to CR2 and CD19 at 145 and 95 kD, respectively, labeled proteins corresponding in molecular mass to p40 and p20 were specifically immunoprecipitated (Fig. 5); a protein at 14 kD was present that had not been observed with ^{125}I labeling. Biosynthetic labeling with [^{35}S]-methionine or a mixture of [^{35}S]-methionine and [^{35}S]-cysteine (data not shown) did not lead to the detection of a 130-kD protein, suggesting a low molar ratio of the ^{125}I - and $^{32}\text{PO}_4$ -labeled protein of this molecular mass relative to the other components of the complex. Assuming comparable half-lives of 14 h for CR2 (41) and CD19 in B lym-

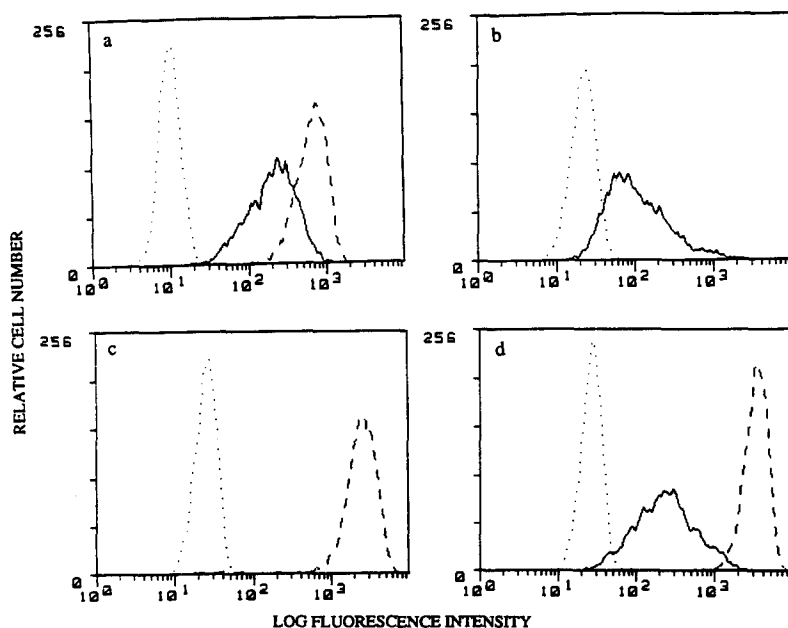


Figure 6. Flow cytometric analysis of Raji cells (a) and K562 cells stably expressing recombinant CR2 (b), recombinant CD19 (c), or CR2 and CD19 (d) that had been indirectly stained with control antibody (dotted line), HB5 anti-CR2 (solid line), or HD37 anti-CD19 (dashed line) followed by fluorescein-conjugated goat anti-mouse Ig.

phoblastoid cells, we determined their molar ratio in the complex by densitometric scanning of the gels, revealing a CD19/CR2 labeling ratio of 0.8. Based on the presence of 12 and 16 methionines in CD19 (36) and CR2 (23, 24), respectively, the molar ratio of these proteins in the complex is calculated to be $\sim 1:1$. This stoichiometry suggests that most molecules of CR2 are contained within complexes with CD19, and that most molecules of CD19 are not associated with CR2.

Reconstitution of the CR2/CD19 Complex in K562 Erythroleukemia Cells. CR2 and CD19 are expressed primarily by B lymphocytes, but the cellular distribution of the other components of the complex is unknown. To determine whether expression of CR2 and CD19 in a cell type other than the B cell would result in the association of these two proteins and in the recovery of the additional components of the complex, K562 erythroleukemia cells were singly or doubly transfected with cDNA constructs encoding CR2 and CD19. The amounts of CR2 and CD19 present on the transfectants were assayed by flow cytometric analysis of cells stained with mAbs and found to be comparable with that on Raji cells (Fig. 6). Only CR2 or CD19 was expressed by the relevant single transfectants, indicating that neither membrane protein requires the other for transport to the plasma membrane; all double transfectants expressed both CR2 and CD19, the latter being present in molar excess over former, as occurs in Raji cells.

Immunoprecipitates prepared with anti-CR2 from digitonin lysates of ^{125}I -labeled, doubly transfected K562 cells revealed the presence not only of CR2 and CD19, but also of proteins of 130, 40, and 20 kD (Fig. 7, lane 1) that were identical in molecular mass to the coprecipitating components of the complex in Raji cells (Fig. 7, lane 7). Thus, the entire complex that had been immunoprecipitated with anti-CR2 from lysates of Raji cells could be recovered from K562 cells by transfection only of CR2 and CD19.

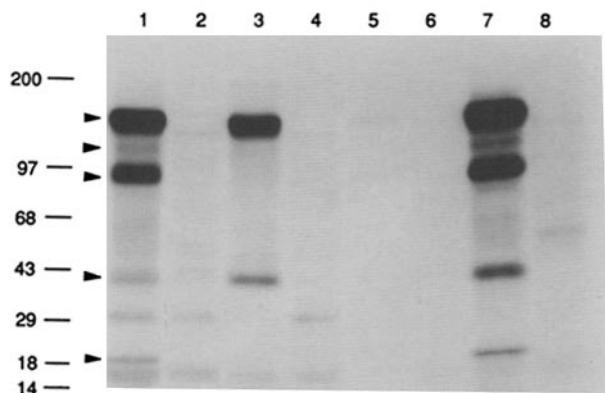


Figure 7. Autoradiograph of SDS-polyacrylamide gel of membrane proteins immunoprecipitated with HB5 anti-CR2 (lanes 1, 3, and 5) and control RPC5.4 IgG2a (lanes 2, 4, and 6), respectively, from digitonin lysates of ^{125}I -labeled K562 cells expressing recombinant CR2 and CD19 (lanes 1 and 2), recombinant CR2 alone (lanes 3 and 4), and recombinant CD19 and (lanes 5 and 6). The immunoprecipitates obtained with anti-CR2 (lane 7) and RPC5.4 IgG2a (lane 8) from the digitonin lysate of ^{125}I -labeled Raji cells are shown for comparison.

Immunoprecipitates prepared with anti-CR2 from digitonin lysates of K562 cells transfected only with CR2 cDNA contained CR2, p130, and p40, but not p20 (Fig. 7, lane 3), indicating that p130 and p40 associate primarily with CR2 and do not require CD19 either for their surface expression or coprecipitation. In contrast, the absence of p20 suggests a primary association with CD19. No specific proteins were recovered with anti-CR2 from lysates of the CD19 single transfectants (Fig. 5, lane 5), providing additional evidence that the proteins coprecipitating with CR2 were not present because of crossreactivity with anti-CR2.

Immunoprecipitates obtained with anti-CD19 from digitonin lysates of the ^{125}I -labeled doubly transfected K562 cells also contained all the components of the complex from Raji cells, except p130, which may have been obscured by the CD19 band, and the 40-kD component (Fig. 8, lanes 1 and 7). The p20 component was enriched in the anti-CD19 immunoprecipitate when compared with its relative amount in the anti-CR2 immunoprecipitate, suggesting that it may associate with CD19. An additional 14-kD protein was present that had not been observed in ^{125}I -labeled immunoprecipitates prepared either with anti-CR2 or anti-CD19 from Raji cells, or with anti-CR2 from the doubly transfected K562 cells. This component was similar in molecular mass to a [^{35}S]methionine-labeled protein coprecipitating with CR2 from Raji cells (Fig. 5).

The possibility that p20 associates with CD19 rather than CR2 was confirmed by analysis of the anti-CD19 immunoprecipitates from the digitonin lysates of ^{125}I -labeled K562 transfectants expressing only CD19; not only was CD19 recovered, but also the p20 and p14 components (Fig. 8, lane 5). A protein of 50 kD also was present in the anti-CD19 immunoprecipitates of K562 cells expressing CD19 alone or with CR2. This component, which probably was present in complexes obtained with anti-CR2 from doubly trans-

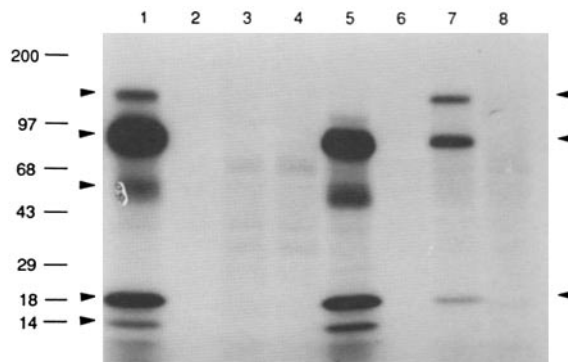


Figure 8. Autoradiograph of SDS-polyacrylamide gel of membrane proteins immunoprecipitated with HD37 anti-CD19 (lanes 1, 3, and 5) and control MOPC21 IgG1 (lanes 2, 4, and 6), respectively, from digitonin lysates of ^{125}I -labeled K562 cells expressing recombinant CR2 and CD19 (lanes 1 and 2), recombinant CR2 alone (lanes 3 and 4), and recombinant CD19 alone (lanes 5 and 6). The immunoprecipitates obtained with anti-CD19 (lane 7) and MOPC21 IgG1 (lane 8) from the digitonin lysate of ^{125}I -labeled Raji cells are shown for comparison.

fecting K562 cells (Fig. 7, lane 1) or Raji cells (Fig. 7, lane 7), also associates with CD19 because it coprecipitated with anti-CD19 from the CD19-expressing K562 cells (Fig. 8, lanes 1 and 5), but not with anti-CR2 from the CR2 single transfectants (Fig. 7, lane 3). The p40 component was absent from anti-CD19 immunoprecipitates, in keeping with its primary association with CR2. In summary, analysis of the K562 transfectants indicates that five membrane proteins that are expressed by this cell type interact with the B cell-specific membrane proteins, CR2 and CD19.

To determine whether the p20 component of B cells resembled that of K562 cells with respect to charge and association with CD19 in the absence of CR2, immunoprecipitates were obtained with anti-CD19 from the CR2⁻ L4 subclone of Ramos cells and from K562 cells that had been singly transfected with CD19 and were subjected to two-dimensional IEF/SDS gels. The CD19 component from both cells was present in the acidic and basic regions of the gel, in which a relatively narrow pH range of 5–7 was used, perhaps reflecting differential glycosylation and phosphorylation (Fig. 9). The p20 component was recovered from the L4 cells, in-

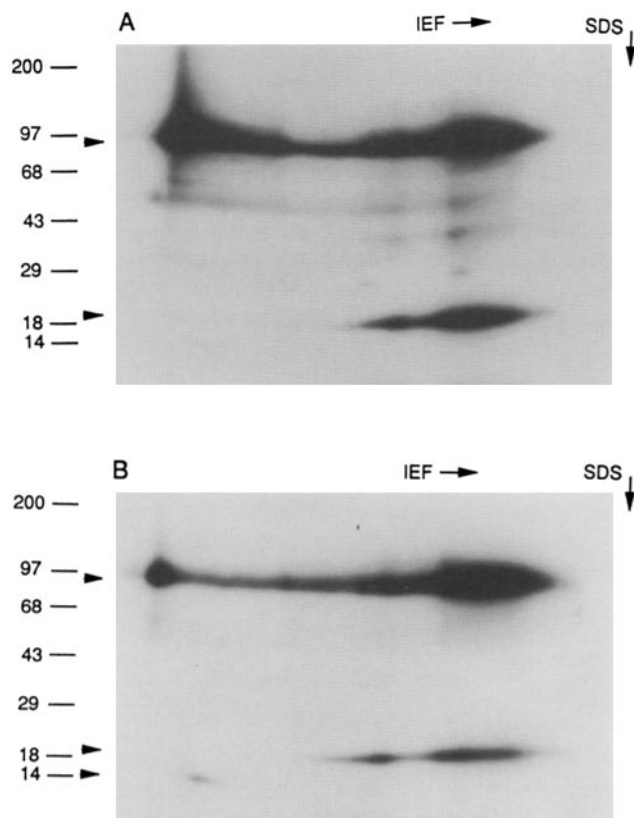


Figure 9. Autoradiograph of the two-dimensional gels (IEF in the first dimension, SDS-polyacrylamide gel in the second dimension) of ¹²⁵I-labeled membrane proteins immunoprecipitated with HD37 anti-CD19 from digitonin lysates of the CR2⁻ L4 subclone of the Ramos Burkitt lymphoma line and of K562 cells expressing recombinant CD19. Arrows indicate positions of CD19, p20, and p14; the last was expressed only in the K562 cell.

dicating its association with CD19 in the B cell lineage, and its pattern on IEF, resembled that of p20 from K562 cells (Fig. 9). Thus, p20 of B cells and K562 erythroleukemia cells are likely to be identical.

Membrane proteins coprecipitating with anti-CR2 and anti-CD19 from ¹²⁵I-labeled Raji cells and K562 cells, respectively, were analyzed by two-dimensional, nonreducing/reducing gels. No components appeared to be disulfide-linked homo- or heterodimers because all proteins fell on the diagonal (Fig. 10).

Crosslinking CD19 to mIgM Synergistically Increases [Ca²⁺]_i, Replicating an Effect of CR2. The function of the complex containing CR2 and CD19 was examined by determining whether CD19 shared with CR2 a capacity for synergistically increasing [Ca²⁺]_i when crosslinked to mIgM on B cells. Replicate samples of Daudi B lymphoblastoid cells that had been loaded with indo-1 were preincubated with optimal and suboptimal concentrations of murine monoclonal anti-IgM, and with saturating concentrations of anti-CR2 and anti-CD19 individually and in combination with suboptimal anti-IgM, respectively. Goat anti-mouse Ig was added to crosslink the cell-bound mAbs, and [Ca²⁺]_i was monitored by flow cytometry. Ligation of mIgM with the higher and lower concentrations of mAb caused eightfold and twofold increments in [Ca²⁺]_i, respectively (Fig. 11). Crosslinking CR2 alone had no effect on [Ca²⁺]_i, whereas crosslinking the receptor to suboptimal amounts of anti-IgM synergistically increased [Ca²⁺]_i by fourfold. Crosslinking CD19 alone caused a two- to threefold increment in [Ca²⁺]_i, and a synergistic rather than additive effect was obtained when CD19 was crosslinked to suboptimal anti-IgM, increasing [Ca²⁺]_i almost to the level seen with cells stimulated with the optimal concentration of anti-IgM.

Daudi B lymphoblastoid cells express an excess of CD19 relative to CR2, so that it is not possible to state whether the CD19-mediated synergy was present in complexes containing or lacking CR2. Although development of CR2- and CD19-deficient B cell lines is required to resolve these two

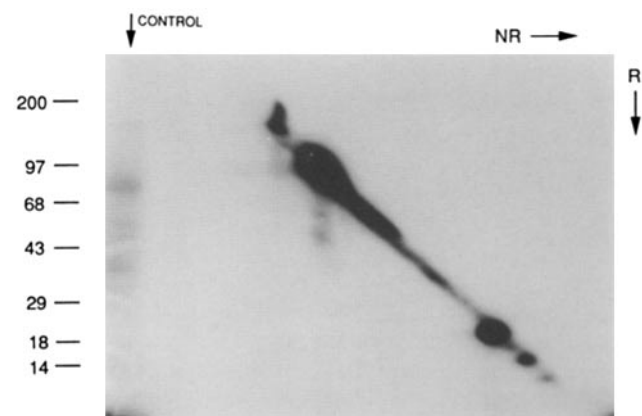


Figure 10. Autoradiograph of two-dimensional gel of membrane proteins immunoprecipitated with HD37 anti-CD19 from a digitonin lysate of ¹²⁵I-labeled K562 cells run in the first dimension without reduction and in the second dimension after reduction of disulfide bonds.

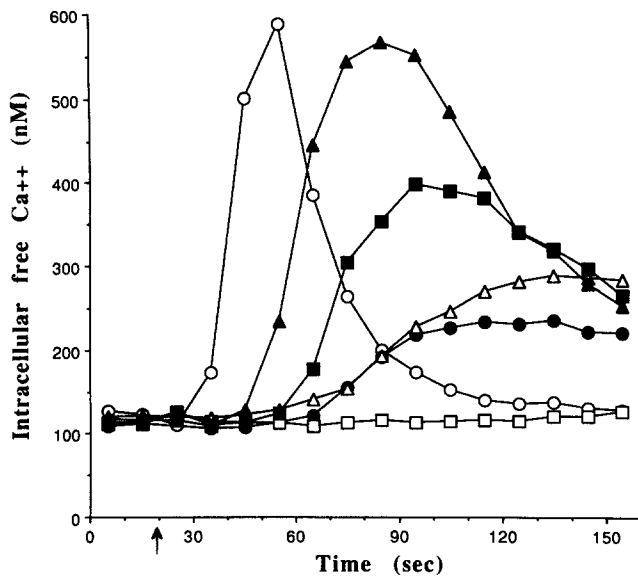


Figure 11. Synergistic increases in $[Ca^{2+}]_i$ induced by crosslinking CR2 and CD19, respectively, to mIgM on Daudi B lymphoblastoid cells. Daudi cells that had been loaded with indo-1 were preincubated with the mAbs Fab DA4.4 anti-IgM at optimal (2 μ g/ml) (○) and suboptimal (0.004 μ g/ml) (●) concentrations, F(ab)₂ HB5 anti-CR2 (□) and IgG1 4G7 anti-CD19 (△), individually and in the combinations, anti-CR2 with suboptimal anti-IgM (■), and anti-CD19 with suboptimal anti-IgM (▲). At the arrow, goat F(ab)₂ anti-mouse Ig was added, and fluorescence was monitored by flow cytometry.

possibilities, the latter may be considered more likely because of the capacity of CD19 to increase $[Ca^{2+}]_i$ when ligated alone and to induce a more marked increase in $[Ca^{2+}]_i$ when ligated to mIgM. With either type of complex, synergistic interaction of CD19 with mIgM suggests that this function of CR2 may reflect its interaction with CD19, which, with its associated components, may be the signal transducer.

Discussion

The present study suggests that the complement system may modulate B cell function through a nonantigen receptor complex that may be representative of a family of receptor complexes found on other cell types. Three findings support this proposal: CR2 is a component of a complex of membrane proteins on the B cell; one of the associated proteins is CD19, a member of the Ig superfamily; and at least one of the other components, p20, interacts with CD19 rather than CR2 and is expressed by the K562 erythroleukemia cell line. The first observation provides a response to the problem that initiated these experiments, which was to understand the mechanism of signal transduction by a membrane protein having only 34 amino acids in its cytoplasmic domain. The second observation provides a potential molecular site of interaction when complement modulates the immune response. The third finding suggests that the CD19 complex of the B cell may be representative of a family of complexes having identical subunits, such as p20, and unique subunits that may be homologues of CD19. The occurrence of such

complexes on non-B cells may indicate that the CD19 complex has an important role in the antigen-independent biology of the B cell, perhaps during development in the bone marrow before expression of mIg.

A complex having the composition, CR2/CD19/p20/(p50)/(p14), designated the CR2/CD19 complex (Fig. 12), was immunoprecipitated with anti-CR2 from digitonin lysates of Raji B lymphoblastoid cells (Figs. 1–3, 8, and 9). Only the first three components are considered to be definite members of the complex. CR2 and CD19 had an ~1:1 molar ratio when antibody to the limiting component, CR2, was used for immunoprecipitation (Fig. 5), and the intensity of indirect iodination of lysines of p20 relative to that of CR2 and CD19 was consistent with substantial amounts of this component in the complex. Also, neither CD19 nor p20 coprecipitated with anti-CR2, nor CR2 and p20 with anti-CD19 in the presence of NP-40 (Fig. 3), making unlikely the possibility that the additional two components were cross-reacting with the immunoprecipitating antibodies or that p20 represented a proteolytic fragment of CD19. The inclusion of the p50 component in the complex is based primarily on its distinct appearance in immunoprecipitates from K562 cells

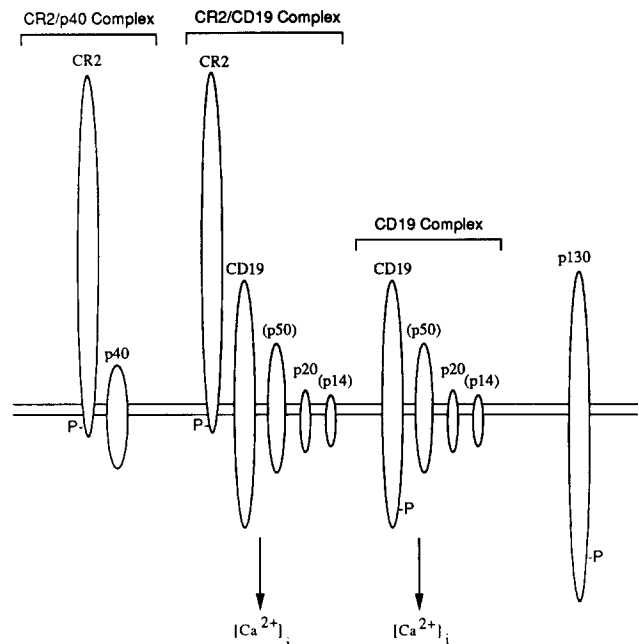


Figure 12. Proposed membrane protein complexes on B lymphocytes containing CR2 and CD19. The CR2/p40 complex is not considered to associate with CD19 because p40 coprecipitated only with anti-CR2 and not with anti-CD19. The CR2/CD19 complex is considered to contain, in addition to these components, p50, p20, and p14. The p50 and p14 components are shown in parentheses because the former has not been clearly identified in Raji cells, and the relationship between the [³⁵S]-methionine-labeled p14 of Raji cells and the [¹²⁵I]-labeled p14 of K562 cells is not known. This complex may augment increases in $[Ca^{2+}]_i$ induced by mIgM. The CD19 complex resembles the CR2/CD19 complex physically and functionally, except for the absence of CR2. The p130 component associates with either CR2 or CD19, but its limited presence in the complexes suggests that it may not be essential for their function.

(Fig. 8), which rendered the faint band in the 50-kD region of the SDS-polyacrylamide gels of immunoprecipitates from Raji cells more plausible (Fig. 7). However, this component, like the constitutive phosphoprotein, p130, which also was not heavily iodinated (Figs. 1-3, 8, and 9) and could not be visualized after labeling with [³⁵S]methionine (Fig. 5), was present in sufficient amounts to justify its definite inclusion in the complex. The p14 component also is not considered a definite member of the complex; in Raji cells it could be labeled only with [³⁵S]methionine (Fig. 5), and its relationship to the ¹²⁵I-labeled p14 of K562 cells is uncertain, especially as the latter apparently could only be immunoprecipitated with anti-CD19 (Figs. 7 and 9). Although its molecular mass is in the range of the γ subunit of the Fc_c receptor type I (Fc_cRI) and of the ζ subunit of the TCR complex, it did not share with these proteins the characteristic of being a disulfide-linked homodimer (Fig. 10).

Primary interactions between the members of CR2/CD19 complex were defined by analysis of the K562 transfectants. Coprecipitating with CD19 from the singly transfected K562 cells expressing this membrane protein were p50, p20, p14, and possibly p130 (Fig. 8), and coprecipitating with CR2 from the CR2 single transfectants were p130 and p40 (Fig. 7). The p40 component, which was also observed to coprecipitate with CR2 in the presence of NP-40 (Fig. 3), is not part of the CR2/CD19 complex, and may be related to functions of CR2 other than modulating [Ca²⁺]_i, such as priming (30), although other explanations for its presence have not been excluded, such as proteolysis of CR2 or alternative splicing of CR2 mRNA. Therefore, CR2 does not bring unique components to the CR2/CD19 complex.

CR2 and CD19 may interact directly in the CR2/CD19 complex, a conclusion that is supported by their 1:1 molar ratio in the complex. This would be the second specific interaction between members of the Ig superfamily and complement system, the previously defined interaction being that between Clq and Ig by which the immune system directs reactions of the complement system. The CR2/CD19 complex differs fundamentally in that it involves membrane rather than soluble proteins, and it may be a mechanism by which complement modulates the immune system.

The finding that CD19 replicated the CR2 function of synergy with mIgM indicates that the CD19 complex with which CR2 associates may mediate this biologic function of the complement receptor (Fig. 11). The possibility that CR2 serves primarily as a ligand binding subunit for the CD19 complex is supported by the additional observation that anti-CD19, which would crosslink approximately fivefold more CD19 complexes than would anti-CR2, increased [Ca²⁺]_i without ligation to mIgM, in accord with earlier reports (47). Although CD19 has been reported to cocap with mIg (48), crosslinking CD19 on a B cell line that lacks mIg also increased [Ca²⁺]_i (data not shown). A signal-transducing function for CD19 in B cells would be consistent with its large intracytoplasmic domain of 247 amino acids (36), its association with p20 and possibly p14, and its presence on pre-B cells, which do not express CR2 or mIg (18, 37). Thus, CR2 may have been grafted onto a pre-existing signal transduction complex of the B cell to link recognition by the complement system to the humoral immune response.

The presence of the p130, p50, p20, and p14 components in the K562 cell may indicate that this cell type has a signal transduction complex containing these proteins, perhaps in association with a component homologous to CD19. The p20 component of the K562 cell and the B cell are likely to be identical; they are similar with respect to size and charge (Fig. 9), and they both associate with CD19 (Figs. 8 and 9). The sharing of components among different signal transduction complexes of different cells of hematopoietic origin has been described for the Fc_cRI of basophils and mast cells (49), the Fc_γ receptor of macrophages (50), and a signal transduction complex of NK cells (51, 52), all of which have the γ subunit first found in association with the Fc_cRI. A corollary to this analysis is that ligand binding components unique to the K562 cell may exist for assembly of a complex that may have some role in the development of cells along the erythrocyte lineage, as, perhaps, does the CD19 complex in development of B cells before expression of mIg. It will be of interest to determine whether other cell types that mature in the bone marrow also express elements of this signal transduction complex.

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