Molecular Interactions of Complement Receptors on B Lymphocytes: A CR1/CR2 Complex Distinct from the CR2/CD19 Complex

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Summary

The complement system augments the humoral immune response to low concentrations of antigen. This effect may be partly mediated by complement receptors on the surface of B lymphocytes that bind immunogenic complexes bearing fragments of C3 and C4. We have shown by immunoprecipitation analysis that the two complement receptors expressed by B lymphocytes, complement receptor 1 (CR1) and CR2, form a detergent-sensitive complex on the surface of tonsillar B lymphocytes and on K562 erythroleukemia cells that were co-transfected with cDNAs encoding CR1 and CR2. The CR1/CR2 complex is distinct from the CR2/CD19 complex and may assist B cell activation by efficiently capturing C3b-containing immunogens and maintaining such immunogens on the B cell after CR1 and factor I–mediated cleavage to iC3b and C3dg. The complement activating immunogen may then trigger signal transduction by the CR1/CR2 complex, the CR2/CD19 complex, or membrane immunoglobulin.

Studies in animals and humans have shown that inherited or experimentally induced deficiencies in components of the classical pathway of complement activation are associated with a diminished humoral immune response to T-dependent and -independent antigens (1–6). This phenomenon may be based on the ability of C3 fragments deposited on a classical pathway activating immune complex to modulate B cell responses to antigen. B cells have two integral membrane proteins that interact with fragments of C3: complement receptor type 1 (CR1, CD35), a 220-kD protein that binds the primary activation fragment of C3, C3b, and serves as a cofactor for its cleavage to iC3b and C3dg, and complement receptor type 2 (CR2, CD21), a 145-kD protein that binds the iC3b and C3dg fragments (7, 8).

CR1 and CR2 have been shown individually to elicit several B cell functions, such as enhancement by ligated CR1 of the differentiation into antibody-secreting cells (9, 10), and augmented cellular proliferation by ligated CR2 in combination with PMA (11), T cell derived factors (12), and anti-IgM (13), respectively. Two studies have compared the two receptors for their participation in defined B cell responses and have suggested that CR2 may have functions not shared by CR1. In the first study, crosslinking of CR2, but not CR1, to membrane IgM on B cells was shown to increase synergistically the intracellular [Ca^{2+}] (14), and, in the second, administration to mice of a mAb reactive with both

CR1 and CR2 was more effective in suppressing the immune response to sheep erythrocytes than was an antibody specific for CR1 (15).

A molecular basis for signal transduction by CR2, and possibly for its unique biological function relative to CR1, has been suggested recently to be its association with a membrane protein complex on the B cell that contains CD19 and several other unidentified components (16). However, the association of CR1 with CD19 could not be assessed in this study because the B lymphoblastoid cell line in which the CR2/CD19 complex was demonstrated did not express CR1. We have now determined the molecular complexes formed by CR1, CR2, and CD19 on normal tonsillar B lymphocytes that express all three proteins and find that CR1 forms a complex with CR2 and that these CR1/CR2 complexes, in contrast to CR2 alone, do not associate with CD19.

Materials and Methods

Monoclonal Antibodies, Cells, and Immunoprecipitations. The anti-CR1 mAbs YZ1 (17) and 3D9 (18), the anti-CR2 mAbs HB5 (19, 20) and OKB7 (Ortho Diagnostic Systems Inc., Westwood, MA), the anti-CD19 mAb HD37 (Dako Corp., Santa Barbara, CA), and W6/32 anti-HLA class I (American Type Culture Collection [ATCC], Rockville, MD) were used. Control antibodies for nonspecific immunoprecipitations were RPC5.4 IgG2a (ATCC) and MOPC21 IgG1 (Cappel Laboratories, Malvern, PA). Tonsillar B

lymphocytes were purified as described (13). K562 erythroleukemia cells were grown in RPMI supplemented with 10% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. Cells were radiolabeled with ^{125}I (21), lysed using either NP40 or digitonin, immunoprecipitated, and analyzed by polyacrylamide gel electrophoresis in the presence of SDS (SDS-PAGE) under reducing conditions (16). Rabbit gammaglobulin (Sigma Chemical Co., St. Louis, MO) bound to CNBr-activated Sepharose (Sigma Chemical Co.) was used to preclear the lysates, and rabbit anti–mouse Ig (Cappel Laboratories) bound to CNBr-activated Sepharose was used for specific immunoprecipitations.

Transfection of K562 Cells with cDNA Constructs. pLNCX.CR2 encodes neomycin resistance and CR2 under transcriptional control of a Maloney murine leukemia virus (MLV) long terminal repeat (LTR) and the CMV immediate early promoter, respectively. It was created by digestion of pLNCX (22) and pBS.CR2.1 (23) with Hind III and Not I, respectively, and treatment of both plasmids with Klenow fragment followed by Cla I digestion and ligation. p141.CD19 confers hygromycin resistance controlled by the HSV tk gene promoter and encodes CD19 driven by the MLV LTR. It was created by digestion of p141 (24) and pZip.neo. SV(CD19) (16) with Hind III and ligation of the appropriate fragments. piABCD encodes the cDNA for CR1 under control of the CMV immediate early promoter (25). K562 cells were transfected and selected as described (16). K562 cells were cotransfected with the expression plasmids for CR2 and CR1, pLNCX.CR2 and piABCD, and selected with neomycin to generate CR1$^+$CR2$^+$K562 cells, or cotransfected with piABCD and the CD19-expressing plasmid, p141.CD19, and selected with hygromycin to generate CR1$^+$CD19$^+$K562 cells, or triply-transfected with piABCD, pLNCX.CR2, and p141.CD19 and selected with both neomycin and hygromycin to generate CR1$^+$CR2$^+$CD19$^+$ K562 cells. Cells expressing high levels of the recombinant proteins were obtained by immunofluorescent staining followed by sorting on a FACStar Plus flow cytometer (Becton Dickinson and Co., Sunnyvale, CA).
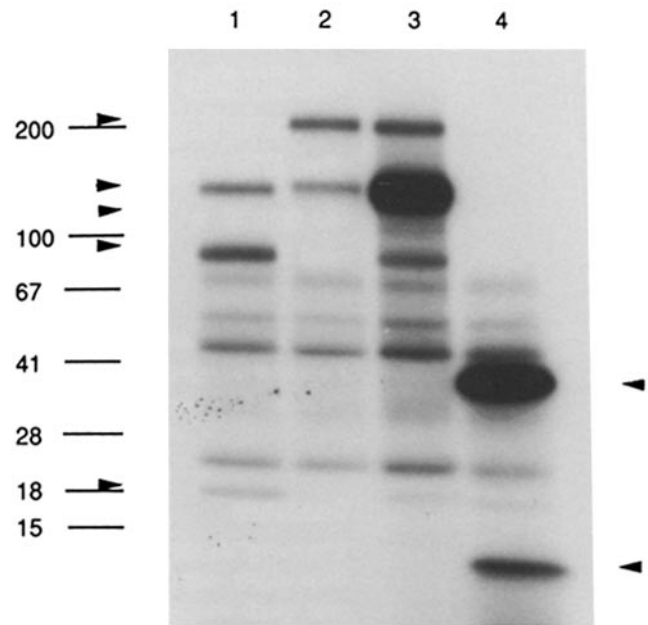

Figure 1. Autoradiograph of SDS-polyacrylamide gel of immunoprecipitates obtained from digitonin lysates of ^{125}I surface-labeled tonsillar B lymphocytes with HD37 anti-CD19 (lane *1*), YZ1 anti-CR1 (lane *2*), HB5 anti-CR2 (lane *3*), and W6/32 anti-HLA (lane *4*), respectively. The arrowheads on the left side and on the right side indicate the positions of specifically immunoprecipitated proteins in lanes *1–3*, and lane *4*, respectively.

Results

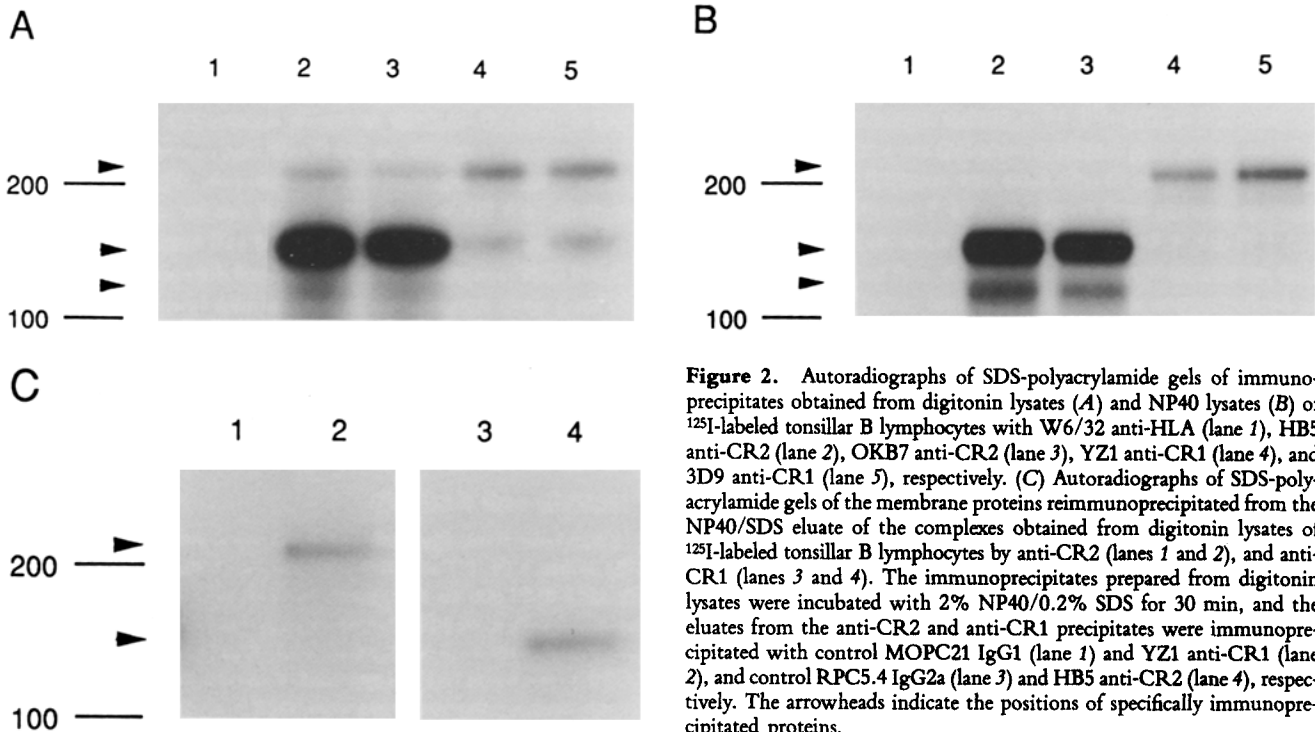*Association of CR1 with CR2 but not CD19 on B Lymphocytes.* To study molecular complexes on B lymphocytes that

Figure 2. Autoradiographs of SDS-polyacrylamide gels of immunoprecipitates obtained from digitonin lysates (*A*) and NP40 lysates (*B*) of ^{125}I-labeled tonsillar B lymphocytes with W6/32 anti-HLA (lane *1*), HB5 anti-CR2 (lane *2*), OKB7 anti-CR2 (lane *3*), YZ1 anti-CR1 (lane *4*), and 3D9 anti-CR1 (lane *5*), respectively. (*C*) Autoradiographs of SDS-polyacrylamide gels of the membrane proteins reimmunoprecipitated from the NP40/SDS eluate of the complexes obtained from digitonin lysates of ^{125}I-labeled tonsillar B lymphocytes by anti-CR2 (lanes *1* and *2*), and anti-CR1 (lanes *3* and *4*). The immunoprecipitates prepared from digitonin lysates were incubated with 2% NP40/0.2% SDS for 30 min, and the eluates from the anti-CR2 and anti-CR1 precipitates were immunoprecipitated with control MOPC21 IgG1 (lane *1*) and YZ1 anti-CR1 (lane *2*), and control RPC5.4 IgG2a (lane *3*) and HB5 anti-CR2 (lane *4*), respectively. The arrowheads indicate the positions of specifically immunoprecipitated proteins.

contain complement receptors, digitonin lysates of ^{125}I-labeled tonsillar B cells were immunoprecipitated with monoclonal anti-CR1, anti-CR2, and anti-CD19, respectively (Fig. 1). Analysis by SDS-PAGE of the immunoprecipitate obtained with anti-CD19 revealed the components of the CR2/CD19 complex: CR2 at 145 kD, CD19 at 95 kD and the unidentified protein at 20 kD, p20 (16) (lane 1). Monoclonal anti-CR1 immunoprecipitated CR1 at 220 kD and another protein at 145 kD having the same mobility as CR2 (lane 2). The immunoprecipitate obtained with anti-CR2 revealed all the components seen with anti-CD19 and anti-CR1: the CR2/CD19 complex and a protein at 220 kD having the same mobility as CR1 (lane 3). The protein at 130 kD coprecipitating with CR2 has been noted previously and has not been identified (16). The control immunoprecipitate of class I HLA revealed the heavy and light chains of class I at 40 kD and 12 kD, respectively, but did not contain any of the specific bands seen in lanes 1, 2, and 3 (lane 4).

To determine whether the complex between the 220-kD and 145-kD proteins was dependent on the type of detergent and the particular monoclonal anti-receptor antibodies used, digitonin and NP40 lysates were prepared from ^{125}I-surface labeled tonsillar B cells, and precipitates were obtained with four different mAbs. Immunoprecipitates from digitonin lysates again revealed the coprecipitation of a 220-kD protein with either of two anti-CR2 mAbs that bind to distinct epitopes, HB5 and OKB7 (23), and the coprecipitation of a 145-kD protein with either of two anti-CR1 mAbs that bind to distinct epitopes, YZ1 and 3D9 (our unpublished observation) (Fig. 2 A). In contrast, immunoprecipitates obtained with these mAbs from NP40 lysates revealed only proteins for which the mAbs were specific (Fig. 2 B).

The differential sensitivity of the complexes to detergents permitted the direct identification of the coprecipitating proteins as CR1 and CR2. Immunoprecipitates were prepared from digitonin lysates with anti-CR1 and anti-CR2, respectively, and treated with NP40 to release any components sensitive to this detergent. Reprecipitation from the NP40 eluates demonstrated that CR1 was specifically recovered from the anti-CR2 immunoprecipitate (Fig. 2 C, lane 2), and that CR2 was recovered from the anti-CR1 immunoprecipitate (Fig. 2 C, lane 4).

Reconstitution of the CR1/CR2 Complex in Transfected K562 Erythroleukemia Cells. In a previous study, the CR2/CD19 complex had been reconstituted in K562 cells by stably expressing recombinant forms of these proteins (16). Having determined that an additional complex of CR1 and CR2 is present on normal B lymphocytes, K562 cells, which do not express CR1 constitutively, were transfected with plasmids containing cDNAs encoding CR1 and CR2 to assess the formation of the CR1/CR2 complex in this cell type. Cell lines expressing both receptors were analyzed by fluorescent flow cytometry (Fig. 3 A), and labeled with [125]I. Immunoprecipitates obtained with anti-CR1 and anti-CR2, respectively, from digitonin lysates of the CR1+CR2+K562 cells demonstrated the presence of the coprecipitating receptor in each instance (Fig. 3 B), indicating the formation of the CR1/CR2 complex. In these experiments, a specific band at 190 kD was

also observed which has not been identified, but may represent a variably glycosylated form of CR1.

To determine whether CR1 could associate with CD19 in the absence of CR2, K562 cells were doubly transfected with cDNAs encoding CR1 and CD19. Cells expressing both proteins (Fig. 4 A) were labeled with ^{125}I, lysed in digitonin and subjected to immunoprecipitation with anti-CR1 and anti-CD19, respectively (Fig. 4 B). Only the protein primarily reacting with the mAb was detected by SDS-PAGE, indicating that in the K562 cell, as had been observed with B
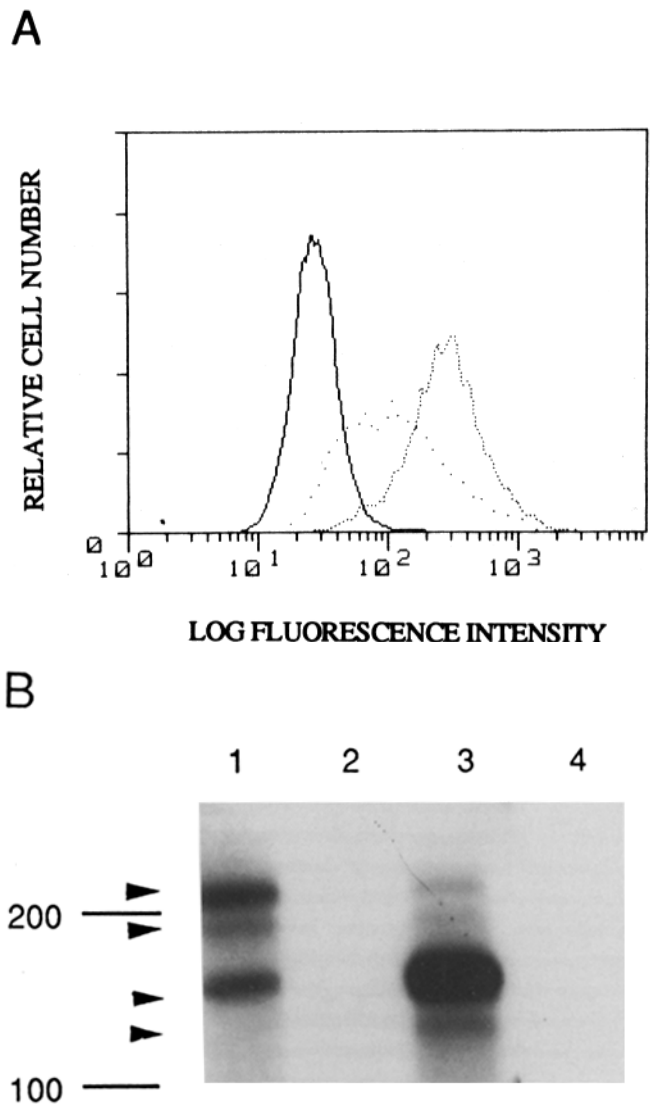

Figure 3. (A) Flow cytometric analysis of K562 cells stably expressing recombinant CR1 and CR2 that had been indirectly stained with control antibody (*solid line*), YZ1 anti-CR1 (*tightly spaced dotted line*), or HB5 anti-CR2 (*loosely spaced dotted line*) followed by fluorescein-conjugated goat anti–mouse Ig. (B) Autoradiograph of SDS-polyacrylamide gel of immunoprecipitates obtained from digitonin lysates of ^{125}I-labeled K562 cells expressing recombinant CR1 and CR2 with YZ1 anti-CR1 (lane 1), control MOPC21 IgG1 (lane 2), HB5 anti-CR2 (lane 3), and control RPC5.4 IgG2a (lane 4), respectively. The arrowheads indicate the positions of specifically immunoprecipitated proteins.
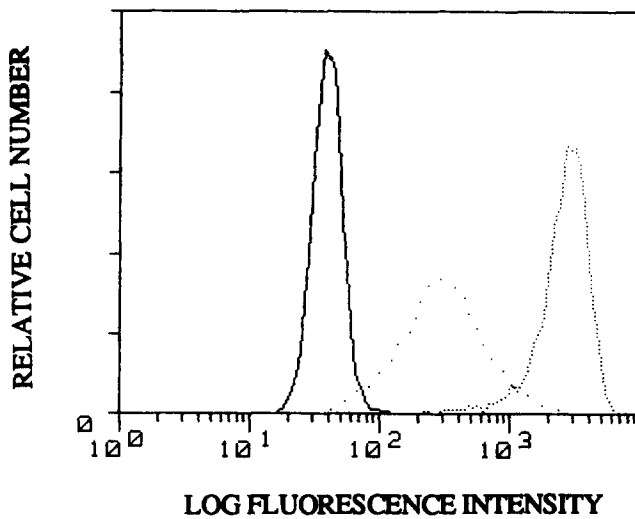
A

B

Figure 4. (*A*) Flow cytometric analysis of K562 cells stably expressing recombinant CR1 and CD19 that had been indirectly stained with control antibody (*solid line*), YZ1 anti-CR1 (*loosely spaced dotted line*), and HD37 anti-CD19 (*tightly spaced dotted line*) followed by fluorescein-conjugated goat anti–mouse Ig. (*B*) Autoradiograph of SDS-polyacrylamide gel of immunoprecipitates obtained from digitonin lysates of ^{125}I-labeled K562 cells expressing recombinant CR1 and CD19 with YZ1 anti-CR1 (lane *1*), control MOPC21 IgG1 (lane *2*), and HD37 anti-CD19 (lane *3*), respectively. The arrowheads indicate the positions of specifically immunoprecipitated proteins.
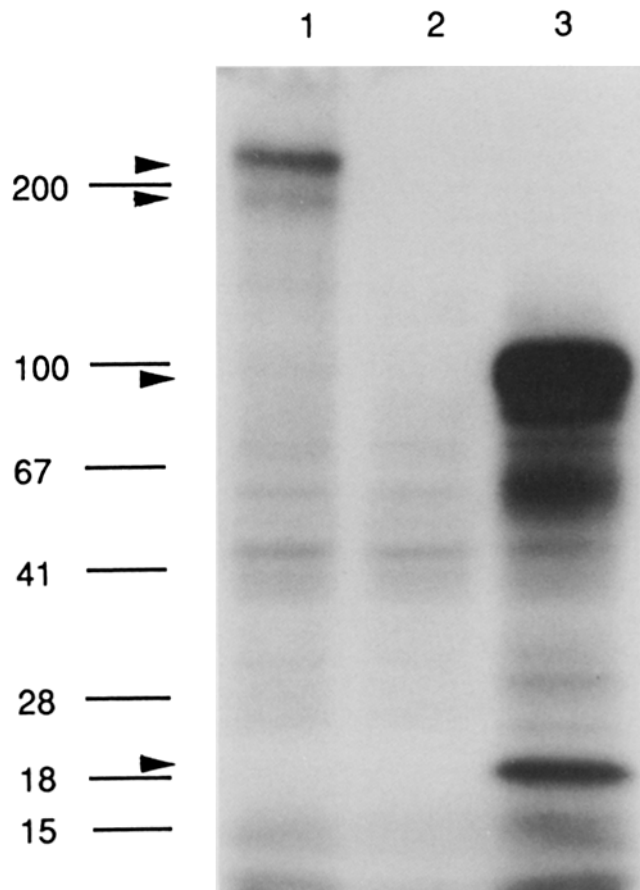
cells, CR1 and CD19 do not form a complex detectable by this assay.

To reconstitute the entire ensemble of complement receptor-containing complexes found on B lymphocytes, K562 cells were triply transfected with cDNAs encoding CR1, CR2, and CD19. Analysis of the triple transfectants by flow cytometry showed expression of all three molecules (Fig. 5 *A*), and immunoprecipitation from digitonin lysates of surface-labeled cells indicated that only the CR1/CR2 complex was obtained with anti-CR1 (Fig. 5 *B*, lane *1*), only the CR2/CD19 complex with anti-CD19 (lane *3*), and both complexes with anti-CR2 (lane *5*). Thus, elements unique to B lymphocytes, other than the three transfected membrane proteins, are not required for the assembly of the complement receptor-containing complexes.

Discussion

A molecular complex containing CR1 and CR2 resides on the surface of B lymphocytes. The CR1/CR2 complex is immunoprecipitated from digitonin, but not NP40, detergent lysates by several mAbs directed against either CR1 or CR2 (Fig. 2, *A* and *B*), indicating that the coprecipitation

is not due to antigenic crossreactivity between these homologous proteins. The coprecipitating proteins were identified as CR1 or CR2 by eluting the immunoprecipitates obtained from digitonin lysates with NP40 and immunoprecipitating the associated receptor from the NP40 eluate with the relevant mAb (Fig. 2 *C*). In some experiments, most of the CR1 appeared to coprecipitate with CR2, suggesting that little or no CR1 was in an uncomplexed state (Fig. 1). Expressing recombinant CR1 and CR2 in K562 erythroleukemia cells reconstituted the CR1/CR2 complex (Fig. 3), indicating that no other lymphoid-specific components were necessary and suggesting that CR1 and CR2 directly interact.

The CR1/CR2 complex is distinct from the CR2/CD19 complex, as is schematically depicted in Fig. 6. Immunoprecipitates of CR1 from B lymphocytes (Fig. 1) or triply transfected K562 cells (Fig. 5) coprecipitated CR2 but not CD19, and, conversely, immunoprecipitates of CD19 from both cell types coprecipitated CR2 but not CR1. Furthermore, the lack of association between CR1 and CD19 is not caused by competition by CR2 because a CR1/CD19 complex also could not be demonstrated on K562 cells expressing these recombinant proteins in the absence of CR2 (Fig. 4). The mutual exclusivity of CR2 for either CR1 or CD19 may be
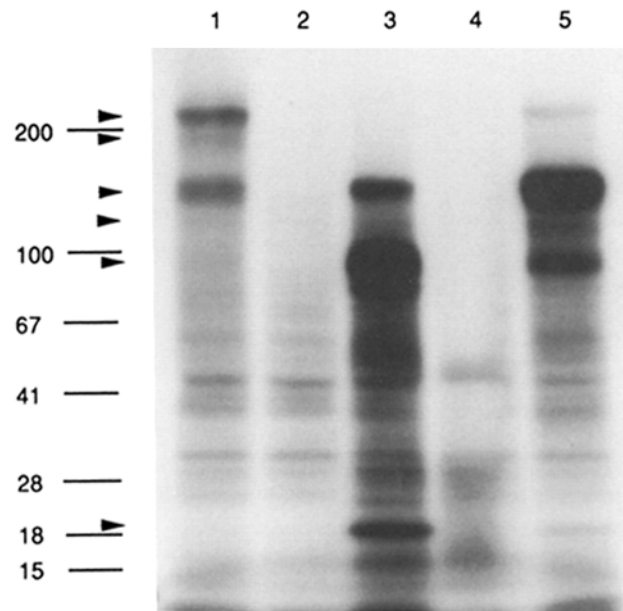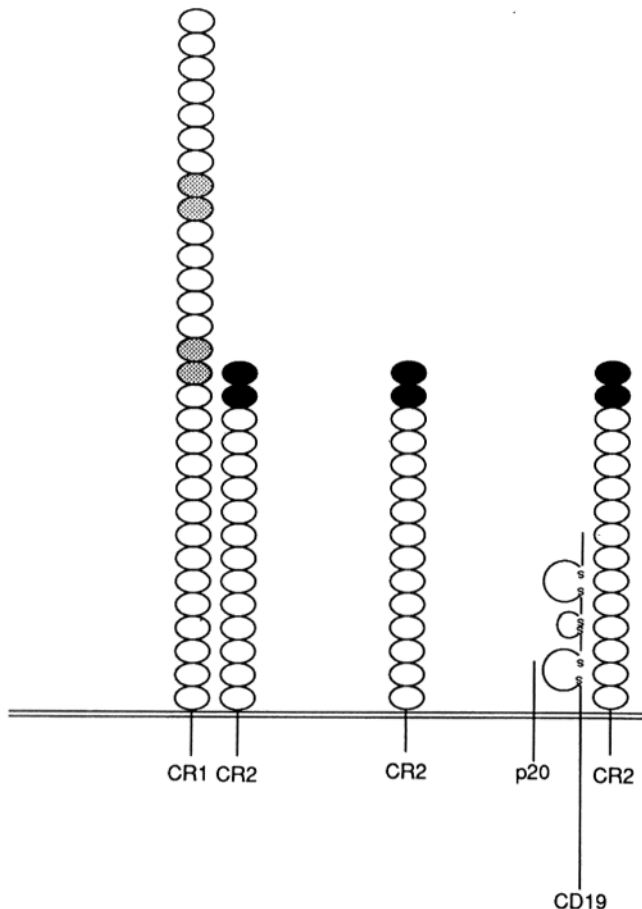
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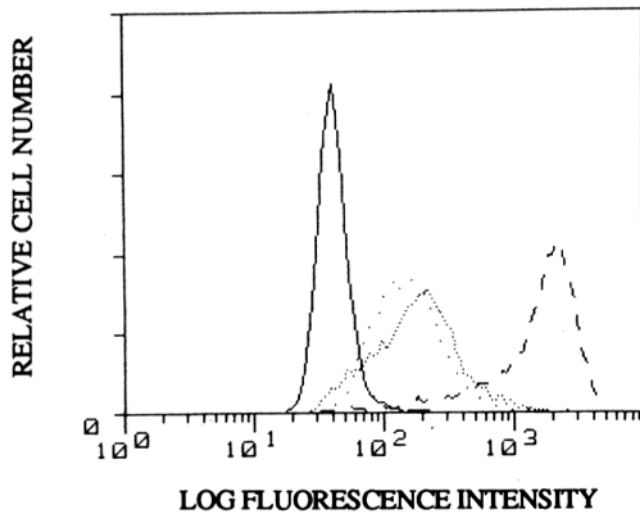
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Figure 5. (A) Flow cytometric analysis of K562 cells stably expressing recombinant CR1, CR2 and CD19 that had been indirectly stained with control antibody (*solid line*), YZ1 anti-CR1 (*tightly spaced dotted line*), HB5 anti-CR2 (*loosely spaced dotted line*), and HD37 anti-CD19 (*dashed line*) followed by fluorescein-conjugated goat anti–mouse Ig. (B) Autoradiograph of SDS-polyacrylamide gel of immunoprecipitates obtained from digitonin lysates of ^{125}I-labeled K562 cells expressing recombinant CR1, CR2, and CD19 with YZ1 anti-CR1 (lane *1*), control MOPC21 IgG1 (lane *2*), HD37 anti-CD19 (lane *3*), control RPC5.4 IgG2a (lane *4*), and HB5 anti-CR2 (lane *5*), respectively. The arrowheads indicate the positions of specifically immuno-precipitated proteins.

Figure 6. Molecular interactions involving complement receptors on B lymphocytes. CR1 and CR2 are comprised of repetitive structural elements termed short consensus repeats (SCRs) (7), and CD19 is a member of the Ig superfamily containing two extracellular Ig domains (32, 33) and associates with p20 (16). The C3b binding sites on CR1 are indicated by shaded SCRs (25), and the iC3b/C3dg binding site on CR2 by closed SCRs (23); a ligand for CD19 has not been identified. The close apposition of the C3b binding sites in CR1 and the iC3b/C3dg binding site in CR2 may facilitate the intracomplex transfer of C3 ligands from CR1 to CR2 after proteolysis by factor I. The mutually exclusive interaction between CR2 and either CR1 or CD19 provides a potential mechanism for the B cell to discriminate between activators of the alternative and classical pathways. Free CR2 may stabilize binding of ligand to either the CR1/CR2 or CR2/CD19 complexes.

the result of a common site on CR2 interacting with both CR1 and CD19, a circumstance that would prevent the formation of a multimolecular complex containing CR1, CR2, and CD19. The apparently greater amount of CR2 obtained by immunoprecipitating with anti-CR2 than with anti-CR1 and anti-CD19 taken together (Fig. 1), suggests that uncomplexed CR2 is present on the membrane, perhaps for the purpose of enhancing multivalent interactions with ligands at the CR1/CR2 and CR2/CD19 complexes, or for preventing the cross-ligation of the CR1/CR2 and CR2/CD19 complexes.

The finding of two distinct complexes that contain complement receptors indicates that an intricate set of interactions may occur between B lymphocytes and immunogenic activators of complement that bear fragments of C3. An ini-

tial function of the CR1/CR2 complex may be the capture of complement activators in which C3 is principally in the C3b form, as would occur with alternative pathway activators (26). The C3b would be converted rapidly to iC3b and C3dg through the concerted actions of CR1 and factor I (27), and the complement activator would transfer preferentially to the associated CR2 rather than to free CR2 or the CR2/CD19 complexes. The association of CR1 with CR2 may also diminish the likelihood that the immunogen would dissociate from the cell following proteolytic processing of C3b. This consideration may be important for immunogen retention on follicular dendritic cells that express both CR1 and CR2 (28), and relate to the proposal that murine CR1 is an extended form of CR2, possibly having CR2 ligand binding capability (29–31).

Ligated CR1/CR2 complex on the B cell may have signal transducing functions, perhaps in relation to cellular differentiation into an antibody-secreting state (9, 10), that are distinct from the CR2/CD19-dependent increase in intracellular $[Ca^{2+}]$ (14, 16). Indeed, the inability of CR1 to associate with CD19 accounts for its lacking this activity of CR2 and further emphasizes the essential role of CD19 in this response. In addition, the CR2 may dissociate from the CR1 with which it is reversibly associated, and diffuse to free CD19 complexes to induce Ca^{2+}-dependent B cell responses. A different series of events may occur when the complement activator bears principally iC3b and C3dg, as would be anticipated for antigen-antibody complexes that have triggered the classical pathway, and in which initial uptake by the B cell would not necessarily be targeted to CR1/CR2 but also to CR2/CD19. In this circumstance, the CD19-dependent response might predominate. Thus, the B cell may be capable of modulating its response to immunogen depending on the mechanism of complement activation because of the presence of distinct CR1/CR2 and CR2/CD19 complexes that may be coupled to distinct signal transduction systems (Fig. 6).

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