Functional Dissection of the CD21/CD19/ TAPA-1/Leu-13 Complex of B Lymphocytes

By Alan K. Matsumoto,* Don R. Martin,* Robert H. Carter,* Lloyd B. Klickstein,§ Joseph M. Ahearn,*‡ and Douglas T. Fearon*‡

*From the *Division of Molecular and Clinical Rheumatology and the Department of Medicine, and the ‡Department of Molecular Biology and Genetics, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205; and the §Center for Blood Research, Harvard Medical School, Boston, Massachusetts 02115*

Summary

The CD21/CD19/TAPA-1 complex of B lymphocytes amplifies signal transduction through membrane immunoglobulin (mIg), recruits phosphatidylinositol 3-kinase (PI3-kinase), and induces homotypic cellular aggregation. The complex is unique among known membrane protein complexes of the immune system because its components represent different protein families, and can be expressed individually. By constructing chimeric molecules replacing the extracellular, transmembrane, and cytoplasmic regions of CD19 and CD21 with those of HLA-A2 and CD4, we have determined that CD19 and TAPA-1 interact through their extracellular domains, CD19 and CD21 through their extracellular and transmembrane domains, and, in a separate complex, CD21 and CD35 through their extracellular domains. A chimeric form of CD19 that does not interact with CD21 or TAPA-1 was expressed in Daudi B lymphoblastoid cells and was shown to replicate two functions of wild-type CD19 contained within the complex: synergistic interaction with mIgM to increase intracellular free calcium and tyrosine phosphorylation and association with the p85 subunit of PI3-kinase after ligation of mIgM. The chimeric CD19 lacked the capacity of the wild-type CD19 to induce homotypic cellular aggregation, a function of the complex that can be ascribed to the TAPA-1 component. The CD21/CD19/TAPA-1 complex brings together independently functioning subunits to enable the B cell to respond to low concentrations of antigen.

The membrane proteins, CD19, TAPA-1, CD21 (complement receptor type 2), and Leu-13 form a complex on human and murine B lymphocytes that has costimulatory activity for the antigen receptor, membrane IgM (mIgM)[1] (1, 2). Impairing the function of this complex in mice by administering antibody to CD21 (3, 4) or a soluble recombinant form of CD21 (5) suppresses complement-mediated enhancement of antibody production to T-dependent antigens. Cross-linking CD19 to mIgM on B cells lowers by two orders of magnitude the number of antigen receptors required for inducing DNA synthesis (6). The required coligation of the complex to the antigen receptor could occur physiologically with antigen that had activated complement to become coated with iC3b and C3dg and capable of interacting with CD21 (for a review, see reference 7), or by cells, such as follicular dendritic and B cells, that may bear antigen on their plasma membranes and express CD23, another ligand for CD21 (8). The absence of CD21 from pre-B cells (9), which express CD19 (10) and TAPA-1 (11), suggests the existence of an additional, as yet unknown, ligand for the complex which would most likely interact with the B cell–specific component, CD19.

The biochemical mechanism by which the complex costimulates is not understood, but may include several effects that have been reported for this membrane entity. Cross-linking any individual component of the complex with specific mAb induces cellular aggregation (12, 13), which may serve to amplify signaling by promoting adhesion of the responding B cell to APCs. Coligating CD21 (14–16), CD19 (1, 2, 17), or TAPA-1 (2) to suboptimal numbers of mIgM synergistically enhances activation of phospholipase C (PLC). Cross-linking CD19 independently of mIgM also activates PLC (18) by a mechanism distinct from that triggered by mIgM (17), and suppresses cellular activation by mIgM (18, 19), the latter effect being reminiscent of that of CD4 on T lymphocytes. CD19 has been shown to be a major substrate of a protein tyrosine kinase activated by mIgM, and tyrosine-phosphory-

[1] *Abbreviations used in this paper:* $[Ca^{2+}]_i$, intracellular free calcium; mIgM, membrane IgM; PI3-kinase, phosphotidylinositol 3-kinase; PLC, phospholipase C; WT, wild-type.

lated CD19 binds phosphatidylinositol 3-kinase (PI3-kinase) (20), an enzyme that is required for the cellular activating effects of certain receptor tyrosine kinases and the polyoma middle T antigen/pp60$^{c\text{-}src}$ complex. Thus, several biochemical pathways may be triggered by the CD21/CD19/TAPA-1/Leu-13 complex that relate to its role in amplifying the response of B cells to antigen.

These multiple biologic effects of the CD21/CD19/TAPA-1/Leu-13 complex may reflect the discrete actions of individual components, each of which is a member of a distinct protein family: CD21 of the regulators of complement activation family (7); CD19 of the Ig superfamily (21, 22); and TAPA-1 of the tetraspan family of membrane proteins (23). Leu-13 has not been structurally characterized. These components also have distinct patterns of cellular expression: CD21 is present on B, follicular dendritic, and some T cells (7); CD19 is found only on B lymphocytes (10); TAPA-1 resides on most cells of hemopoietic lineage (11); and Leu-13 can be expressed by B and T lymphocytes, endothelial cells, and placental trophoblasts (24–26). Thus, the complex differs from certain others of the immune system, such as the TCR–CD3 complex (27) and the mIg/Ig-α,β complex (28, 29), in that its components are derived from several protein families and may be expressed individually, suggesting that each has a unique function in the complex. CD21 has a short, 34-amino acid cytoplasmic domain and an extensive extracytoplasmic region in which are binding sites for iC3b, C3dg (30, 31), and CD23 (8), consistent with a role as a ligand binding subunit to link the complex to fluid phase reactions involving complement, and to cellular reactions associated with the low affinity FcεRII. Signaling by the complex is more likely to involve TAPA-1 and CD19. The former is unusual in having four, highly conserved, transmembrane domains, two of which contain acidic amino acids (23), and the latter has a long cytoplasmic domain of 243 amino acids (22) which already has been shown to participate in the recruitment of PI3-kinase (20). However, it is not known which biologic functions of the complex require CD19, TAPA-1, or both.

The present study defined the domain of CD19 that is required for its interaction with TAPA-1. This permitted the construction of a chimeric CD19 lacking this domain that would reside outside the CD21/CD19/TAPA-1 complex when expressed in B lymphoblastoid cells. This form of CD19 was compared on the same B cell line with wild-type CD19, which was associated with CD21 and TAPA-1, for synergistic enhancement of B cell activation by mIgM, tyrosine phosphorylation, and binding of PI-3 kinase after cross-linking of mIgM, and homotypic cellular aggregation.

Materials and Methods

Antibodies. HB5 anti-CD21 (9) (American Type Culture Collection [ATCC], Rockville, MD), YZ-1 anti-CD35 (32), HD37 anti-CD19 (Dako Corp., Santa Barbara, CA), and B4 anti-CD19 (Cappel Laboratories, Durham, NC), OKT4 anti-CD4 (ATCC), DA4.4 anti-IgM (ATCC), 5A6 anti-TAPA-1 (11) (generous gift of Dr. Shoshanna Levy, Stanford University, Stanford, CA), 4G10 antiphosphotyrosine (UBI, Lake Placid, New York), and anti-p85 (UBI) were used. Isotype-matched control murine antibodies were RPC5.4 IgG2A (ATCC), MOPC21 IgG1 (Cappel Laboratories), and IgG2b (Southern Biotechnology Associates, Birmingham, AL).

Chimeric CD19 Constructs. To replace the extracytoplasmic region of CD19 with that of CD4, and the transmembrane and cytoplasmic regions with HLA-A2, a strategy using PCR primers spanning the chimeric junctions was employed (see Table 1). PCR fragments joining the different domains bounded by unique restriction sites, BstEIII in the extracytoplasmic region of CD4 and ScaI, BsmI, and XhoI in the CD19 extracytoplasmic, transmembrane, and 3′ untranslated regions, respectively, were generated. Chimeric cDNA constructs were cloned into the eukaryotic expression vector, pZipNeoSV (33) by means of flanking BamHI sites. In CD19ΔEc, which replaces the extracytoplasmic region of CD19 with that of CD4, primers CD19.1 and CD19.2 (Table 1) were used on a CD4 template to generate a PCR fragment that contained CD4 sequence from the BstEII site to methionine 374 that was joined to CD19 sequence from valine 276 of the transmembrane domain to the BsmI site. This fragment was cloned into the BstEII site of CD4 and the BsmI site of CD19. CD19ΔCyt1 replaces the CD19 cytoplasmic region with that of HLA-A2 after proline 299, and CD19ΔCyt2 after leucine 303. Using primers CD19.3 and CD19.4 or CD19.5 and CD19.4 on a HLA-A2 cDNA template, two PCR fragments were generated containing the BsmI site, CD19 transmembrane sequence to either proline 299 or leucine 303, HLA-A2 cytoplasmic sequence starting at arginine 293 to the stop codon, and a XhoI site. These fragments were used to replace the BsmI/XhoI fragment of wild-type CD19.

In CD19ΔTmCyt, which replaces both the transmembrane and cytoplasmic region of CD19 with that of HLA-A2, primers 19.6 and 19.4 were used on a HLA template generating a fragment encoding the extracytoplasmic region of CD19 from the ScaI site to lysine 275 joined to the entire HLA transmembrane and cytoplasmic regions at valine 269. This fragment was used to replace the ScaI/XhoI fragment in wild-type CD19 cDNA. In CD19ΔEcCyt, which contains only the CD19 transmembrane region, the BamHI/BsmI fragment of CD19ΔEc containing extracellular CD4 was ligated by the BsmI site to the BsmI/BamHI fragment of CD19ΔCyt2 containing HLA A2 cytoplasmic region. The resulting chimera contains CD4 extracellular, CD19 transmembrane, and HLA cytoplasmic regions.

CD21 Constructs. To construct CD21 mutants which replace the transmembrane and cytoplasmic regions of CD21 with HLA-A2 or delete the cytoplasmic region, a PCR strategy similar to that used with the CD19 chimeras was employed. A unique BglII site was generated in the extracytoplasmic region of CD21 using oligonucleotide-directed mutagenesis and two-step PCR (34). Primers 21.1 and 21.2 were used on a CD21 cDNA template, and the PCR product used together with primer 21.3 on a CD21 template to create a silent C to T transition in the third position of the serine 969 codon. Additional unique restriction sites, EcoRV and BclI in CD21 were used. CD21 chimeras were cloned into the eukaryotic expression vector LXSN (35). In CD21ΔCyt, which replaces the cytoplasmic region of CD21 with HLA-A2, primers 21.5 and 21.6 were used on HLA-A2 template to create a PCR fragment containing the EcoRV site, CD21 transmembrane sequence to serine 978, HLA-A2 cytoplasmic sequence from arginine 293, and the BclI site. This fragment replaced the EcoRV/BclI fragment of wild-type CD21. In CD21ΔTmCyt, which replaces the transmembrane and cytoplasmic regions of CD21 with HLA-A2, primers 21.7 and 21.6 were used on HLA-A2 template to create a fragment joining the CD21 extracellular region at proline 954

to HLA-A2 transmembrane at threonine 265 bounded by BglII and BclI sites.

In CD21 Cyt minus, which deletes the cytoplasmic region of CD21, primers 21.1 and 21.4 were used on a CD21 template to introduce a stop codon after histidine 980 in the cytoplasmic region of CD21.

CD35 Constructs. The CD35 chimeric construct and the CD35 cytoplasmic deletion mutant were created by site-directed mutagenesis performed by the method of Kunkel (36). Constructs were cloned into the eukaryotic expression vector AprM8. In CD35 Cyt minus which deletes the cytoplasmic domain of CD35, oligonucleotide 35.1 was used to introduce a stop codon after histidine 1957 in the cytoplasmic domain of CD35. This leaves only lysine and histidine in the CD35 cytoplasmic domain. In CD35ΔTmCyt, which replaces the transmembrane and cytoplasmic region of CD35 with that of HLA-A2, oligo 35.2 was used to create a unique ApaLI site just 5′ of the HLA transmembrane region. The ApaLI site near the CD35 extracellular/transmembrane junction was used to join the extracellular portion of CD35 at alanine 1928 to the transmembrane/cytoplasmic region of HLA-A2 at glutamine 263.

Cell Lines and Transfections. K562 erythroleukemia cells and Daudi B lymphoblastoid cells were grown in RPMI 1640 supplemented with 10% bovine calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. K562 cells stably expressing wild-type transfected CD19, CD21, or CD35 have been previously described (1). Mutant constructs were introduced into K562 cells by lipofection (1), and stable transfectants selected with 400 μg/ml G418 (Gibco BRL, Gaithersburg, MD) or when cotransfected with plasmid p141, which encodes hygromycin resistance, selected with 400 μg/ml hygromycin B (Calbiochem-Novabiochem Corp., La Jolla, CA). High expressing cells were sorted by fluorescent flow cytometry.

Daudi cells were transfected by electroporation in which 8×10^6 cells were pelleted and brought up in 0.8 ml RPMI with 10% calf serum, and loaded into a 4-mm width electroporation cuvette together with 50 μg linearized DNA. Cells were electroporated at 250 V, 960 μF using a Gene Pulser apparatus (Bio-Rad Laboratories, Richmond, CA). After 24 h of culture, cells were counted, diluted to 10^5, and cultured in media containing G418, 2 mg/ml, in 24-well plates. Clones were isolated and stained for expression by fluorescent flow cytometry.

Immunoprecipitation and Immunoblotting. For analysis of coprecipitating proteins, Daudi cells and K562 transfectants were surface labeled with ^{125}I (New England Nuclear, Boston, MA) (37). Cells were lysed in a buffer containing 1% digitonin, and lysates immunoprecipitated with 5 μg mAbs followed by protein A–trisacryl for isotypes IgG2a and IgG2b, or rabbit IgG anti–mouse Ig bound to Sepharose for IgG1 (1). Proteins were resolved on 5–20% SDS-polyacrylamide gradient gels and detected with autoradiography. To assess tyrosine phosphorylation and association with the p85 subunit of PI3-kinase, Daudi cells were stimulated with $F(ab')_2$ goat anti–human IgM for 5 min at room temperature, pelleted, and lysed in buffer containing 1% NP-40 and phosphatase and protease inhibitors (20). Lysates were precipitated with OKT4 anti-CD4 or HD37 anti-CD19 followed by protein A–trisacryl beads or rabbit anti–mouse Ig bound to Sepharose, respectively. Proteins were resolved on 7.5% SDS-PAGE slab gels, transferred to nitrocellulose, and probed with ^{125}I-labeled 4G10 antiphosphotyrosine antibody. The blots were reprobed with anti-p85 followed by detection by enhanced chemiluminescence (ECL) (Amersham, Arlington Heights, IL).

Analysis of $[Ca^{2+}]_i$ and Homotypic Aggregation of Daudi Cells. Daudi transfectants loaded with indo-1 were preincubated with appropriate mAbs for 15 min at room temperature, washed, and the bound mAbs cross-linked with $F(ab')_2$ goat anti–mouse Ig in the presence of DMSO alone or containing 75 nM PMA.

Table 1. *Oligonucleotides used in preparing CD21, CD19, and CD35 constructs*

Name	5′–3′ Sequence
19.1	GCGTCTAGAGGTTACCCAGGACCCTAAGCTC
19.2	GCGGAATTCGAATGCCCACAAGGGAACACAGGCAGAAGATCAGA TAAGCCAAAGTCACAGCTGAGCCATTGGCTGCACCGGGGTGGA
19.3	GCGTCTAGAGGCATTCTTCATCTTCAAAGGAGGAAGAGCTCAGATAGA
19.4	GCGGAATTCCTCGAGTCACACTTTACAAGCTGTGAG
19.5	GCGTCTAGAGGCATTGTTCATCTTCAAAGAGCCCTGGTCCTGAGG AGGAAGAGCTCAGATAGA
19.6	GCGTCTAGACCAGTACTATGGCACTGGCTGCTGAGGACTGGTGGCTGG AAGGTGGGCATCATTGCTGGCCTG
21.1	TGCAGATCTCGTTCACTTGCTCCTGTC
21.2	TGAATCGATAGCAGTTTCTTTCTAATC
21.3	GTTCACGTGTGGAGCTAG
21.4	CAGTGATCAGTGTTTTGATATCACGTA
21.5	CGTGATATCAAGGAGGAAGAGCTCAGAT
21.6	TTCTGATCACACTTTACAAGCTGT
21.7	TGCAGATCTCGTTCACTTGCTCCTACCATCCCCATCGTGGGC
35.1	ATAATTCTAAAGCACTGAGAAGGCAATAATGCA
35.2	CTGGGATGGGAGCCGTGTGCACAGTCCACCGTCCCC

Changes in intracellular free calcium ($[Ca^{2+}]_i$) were monitored by flow cytometry (17).

Daudi cell transfectants were suspended in RPMI 10% calf serum at 10^7/ml. The mAbs, 5A6 anti-TAPA-1, HD37 anti-CD19, and OKT4 anti-CD4, were diluted in Hanks' buffered saline with 20 mM EDTA and 0.1% BSA. In a 96-well flat bottomed plate (Costar Corp., Cambridge, MA), 0.1 ml of antibody dilution was added to 0.1 ml of cells and the cells incubated at 37°C for 45 min after which photomicrographs of representative fields were taken.

Results

Domains of CD19 that Mediate Association with TAPA-1 and CD21. To determine which regions of CD19 mediate the association of this protein with TAPA-1 and CD21, we prepared constructs encoding chimeric forms of CD19 in which the extracellular, transmembrane, and cytoplasmic regions had been replaced with the corresponding regions of CD4 and HLA A2 (Fig. 1). Chimeric constructs and deletion mutants encoding altered forms of CD21 were also prepared to confirm the results obtained with the CD19 constructs (Fig. 1). Wild-type and mutated constructs were stably transfected into K562 erythroleukemia cells which constitutively express TAPA-1, and incorporation of the membrane proteins into complexes was assessed by analyzing immunoprecipitates obtained with antibodies to CD19, CD4, and CD21 from digitonin lysates of ^{125}I surface-labeled cells. Anti-TAPA-1 could not be used for these studies because it does not coimmunoprecipitate other components of the CD21/CD19/TAPA-1/Leu-13 complex (2) (data not shown), which may reflect disruption of the complex by antibody or masking of its epitope by CD19.

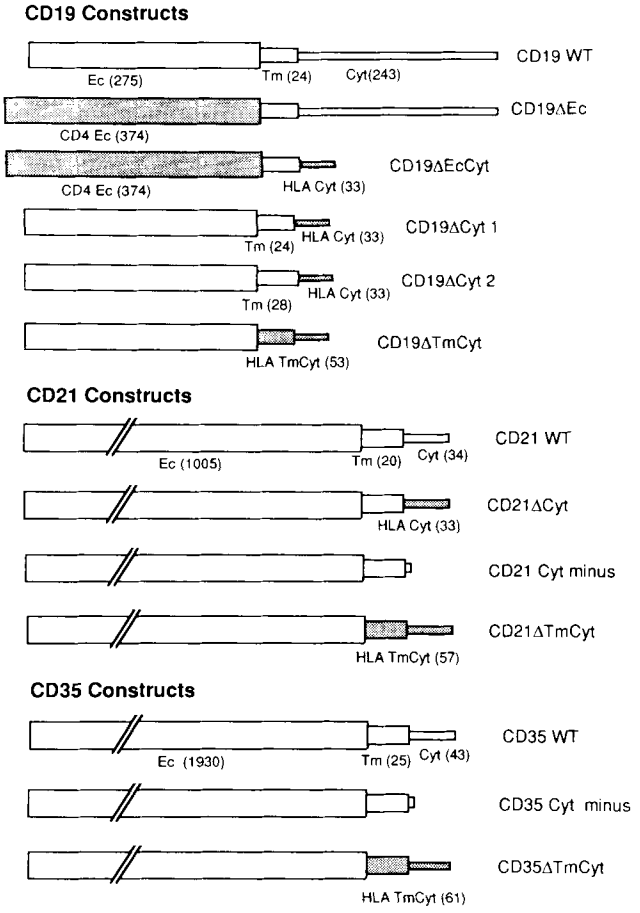

Figure 1. Diagram of wild-type (*WT*) CD19, CD21, CD35, and the mutant constructs in which the extracellular (*Ec*), transmembrane (*Tm*), or cytoplasmic (*Cyt*) regions have been replaced with corresponding segments of HLA-A2 and CD4, or the cytoplasmic regions have been deleted (*Cyt minus*). The shaded portions represent chimeric substitutions and the numbers in parentheses represent the number of amino acids in each domain.

Immunoprecipitation with anti-CD19 of digitonin lysates of K562 cells that had been stably transfected with constructs encoding wild-type CD19 and CD21 recovered not only CD19, but also CD21, TAPA-1, and Leu-13 (Fig. 2 *A*), as has been previously reported (1, 2). Digitonin lysates were prepared of K562 cells expressing wild-type CD21 and either of the chimeric CD19 constructs, CD19ΔEc, in which the extracellular region of CD19 had been replaced with that of CD4, and CD19ΔEcCyt, which also had the cytoplasmic domain replaced with that of HLA-A2, leaving only the transmembrane domain of CD19. Anti-CD4 immunoprecipitated the chimeric proteins at 95 and 65 kD, respectively, and Leu-13, but not CD21 or TAPA-1 (Fig. 2 *A*). The absence of an association of CD21 with CD19ΔEc and CD19ΔEcCyt was observed also when CD21 was primarily immunoprecipitated from lysates of K562 cells expressing these chimeric forms of CD19 (Fig. 2 *B*), confirming the requirement for the extracellular region of CD19 for interaction with both TAPA-1 and CD21.

Because of ambiguity in the position of the transmembrane–cytoplasmic junction in CD19, we prepared two CD19/HLA-A2 chimeras: CD19ΔCyt1, in which the cytoplasmic domain after proline 299 was exchanged with that of HLA-A2, and CD19ΔCyt2, in which the replacement occurred after leucine 303 (Fig. 1). To assess the role of the transmembrane region of CD19, a third construct, CD19-ΔTmCyt, replaced both the transmembrane and cytoplasmic regions of CD19 with those of HLA-A2 (Fig. 1). These were expressed in K562 cells and examined for association with TAPA-1 and CD21. TAPA-1 coimmunoprecipitated with each chimeric form of CD19, indicating that the extracellular region is not only necessary but sufficient to mediate association of CD19 with TAPA-1 (Fig. 3 *A*). CD21 coimmunoprecipitated with the CD19ΔCyt1 and CD19ΔCyt2 mutants, but not with the CD19ΔTmCyt chimera. This requirement for the transmembrane region of CD19 for its association with CD21 was confirmed when immunoprecipitates were obtained with anti-CD21 rather than with anti-CD19 (Fig. 3 *B*). Thus, both the extracellular and transmembrane regions of CD19 appear to mediate the interaction of this protein with CD21.

Further support for this conclusion was obtained by analysis of chimeric forms of CD21 in which the cytoplasmic domain (CD21ΔCyt), or both the transmembrane and cytoplasmic domains (CD21ΔTmCyt) were replaced with these regions from HLA-A2 (Fig. 1). A third construct, CD21 Cyt

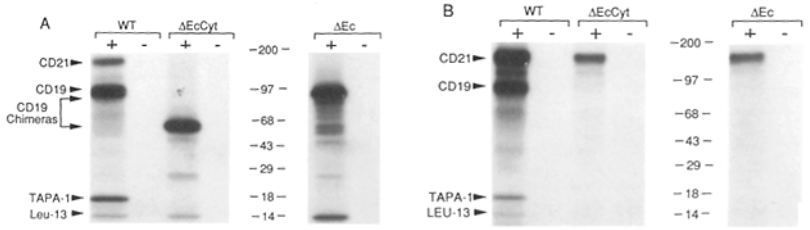

Figure 2. Effects of substituting the extracellular domain of CD19 on its association with CD21 and TAPA-1. (*A*) Digitonin lysates were prepared from ^{125}I-labeled K562 transfectants expressing CD21 WT together with CD19ΔEcCyt or CD19ΔEc. Specific immunoprecipitates (+) of CD19 were obtained with HD37 anti-CD19, and of CD19ΔEcCyt and CD19ΔEc with OKT4 anti-CD4; nonspecific immunoprecipitates (−) were also obtained. The proteins were resolved on SDS-polyacrylamide gels and detected by autoradiography. (*B*) Immunoprecipitates were obtained with HB5 anti-CD21 from replicate digitonin lysates of the ^{125}I-labeled K562 transfectants expressing CD21 WT together with CD19 WT, CD19ΔEcCyt, or CD19ΔEc. The proteins were resolved on SDS-polyacrylamide gels and detected by autoradiography.

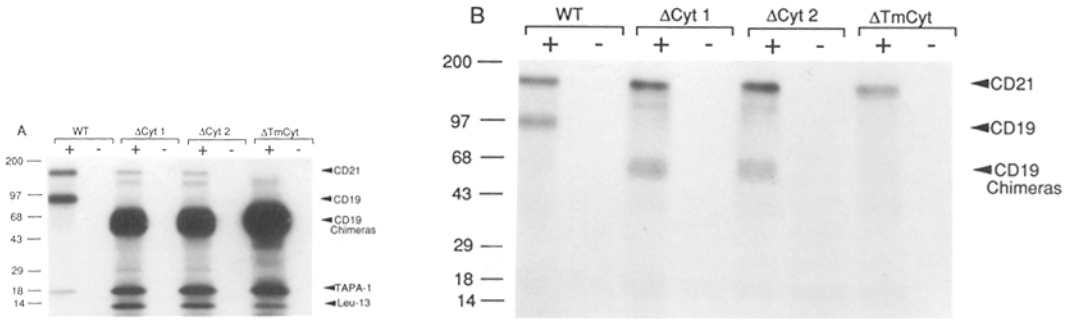

Figure 3. Effect of substituting the transmembrane or cytoplasmic domains of CD19 on its association with CD21 and TAPA-1. (*A*) Digitonin lysates were prepared from ^{125}I-labeled K562 transfectants expressing CD21 WT together with CD19ΔCyt1, CD19ΔCyt2, or CD19ΔTmCyt, and from ^{125}I-labeled Raji B lymphoblastoid cells which bear wild-type CD21 and CD19. Specific immunoprecipitates (+) were obtained from wild-type CD19 and the CD19 chimeras with HD37 anti-CD19. Nonspecific immunoprecipitates (−) were also obtained. The proteins were resolved on SDS-polyacrylamide gels and detected by autoradiography. (*B*) Immunoprecipitates were obtained with HB5 anti-CD21 from replicate digitonin lysates of ^{125}I-labeled K562 transfectants expressing CD21 WT together with CD19ΔCyt1, CD19ΔCyt2, or CD19ΔTmCyt, and from ^{125}I-labeled Raji cells. The proteins were resolved on SDS-polyacrylamide gels and detected by autoradiography.

minus, deleted the cytoplasmic domain of CD21 (Fig. 1). Immunoprecipitation with anti-CD21 from K562 cells transfected with wild-type CD19 and the CD21 constructs indicated that substitution or removal of the cytoplasmic region of CD21 did not alter the coprecipitation of wild-type CD19, whereas substitution of the cytoplasmic and transmembrane domain abolished this association (Fig. 4).

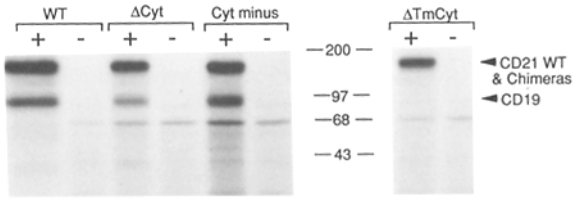

Figure 4. Effect of substituting or deleting the transmembrane or cytoplasmic regions of CD21 on its association with CD19. Digitonin lysates were prepared from ^{125}I-labeled K562 transfectants expressing CD19 WT together with CD21 WT, CD21ΔCyt, CD21ΔTmCyt, or CD21 Cyt minus. Specific immunoprecipitates (+) were obtained with HB5 anti-CD21. Nonspecific (−) immunoprecipitates were also obtained. The proteins were resolved on SDS-polyacrylamide gels and detected by autoradiography.

Domains that Mediate the Association of CD21 and CD35. The CD21ΔTmCyt, CD21ΔCyt, and CD21 Cyt minus constructs were coexpressed with wild-type CD35 in K562 cells to determine the regions mediating the CD21/ CD35 complex (38). Immunoprecipitation with anti-CD35 from digitonin lysates of these cells recovered CD35 and each form of CD21 (Fig. 5), indicating that the transmembrane and cytoplasmic domains of CD21 are not required for interaction with CD35. Consistent with this conclusion was the observation that CD21 was coprecipitated with anti-CD35 from lysates of K562 cells expressing wild-type CD21 together with wild-type CD35, CD35 in which the transmembrane and cytoplasmic domains had been replaced with those of HLA-A2, or CD35 in which the cytoplasmic region had been deleted (Figs. 1 and 6).

Functions Mediated by CD19ΔEc Independent of the CD21/ CD19/TAPA-1 Complex. To determine which functions of CD19 on B cells were dependent on interactions with TAPA-1 or CD21, we expressed the CD19ΔEc chimera, in which the extracellular domain had been replaced with that of CD4, in Daudi B lymphoblastoid cells which also express constitutively wild-type CD19, TAPA-1, and CD21. Daudi cells do not express Leu-13 and any functions mediated by

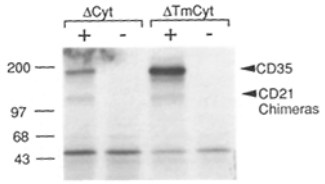

Figure 5. Effect of substituting the transmembrane or cytoplasmic regions of CD21 on its association with CD35. Digitonin lysates were prepared from ^{125}I-labeled K562 transfectants expressing CD35 WT together with CD21ΔCyt or CD21ΔTmCyt. Specific immunoprecipitates (+) were obtained with YZ-1 anti-CD35. Nonspecific immunoprecipitates (−) were also obtained. The proteins were resolved on SDS-polyacrylamide gels and detected by autoradiography.

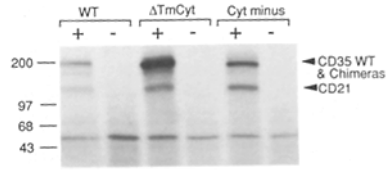

Figure 6. Effect of substituting or deleting the transmembrane or cytoplasmic region of CD35 on its association with CD21. Digitonin lysates were prepared from ^{125}I-labeled K562 transfectants expressing CD21 WT together with CD35ΔTmCyt or CD35 Cyt minus. Specific immunoprecipitates (+) were obtained with YZ-1 anti-CD35. Nonspecific immunoprecipitates (−) were also obtained. The proteins were resolved on SDS-polyacrylamide gels and detected by autoradiography.

CD19ΔEc in this cell line would be independent of Leu-13. The amount of the chimeric protein expressed by these cells was approximately one half that of wild-type CD19. Immunoprecipitates obtained with anti-CD4 from transfected Daudi cells contained only the CD19ΔEc chimera, whereas anti-CD19 recovered wild-type CD19, CD21, and TAPA-1 from replicate samples of lysates (Fig. 7).

The capacity of the CD19ΔEc chimera synergistically to increase $[Ca^{2+}]_i$ when coligated with suboptimal numbers of mIgM on transfected Daudi cells was compared with that of wild-type CD19, which is contained within the complex,

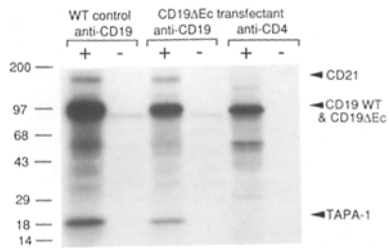

Figure 7. Analysis of membrane proteins coimmunoprecipitating with wild-type CD19 and recombinant CD19ΔEc from transfected Daudi cells. Digitonin lysates were prepared from ^{125}I-labeled Daudi transfectants expressing CD19ΔEc, and from ^{125}I-labeled wild-type Daudi cells. Specific immunoprecipitates (+) were obtained of wild-type CD19 with HD37 anti-CD19, and of CD19ΔEc with OKT4 anti-CD4; nonspecific immunoprecipitates (−) were also obtained. The proteins were resolved on SDS-polyacrylamide gels and detected by autoradiography.

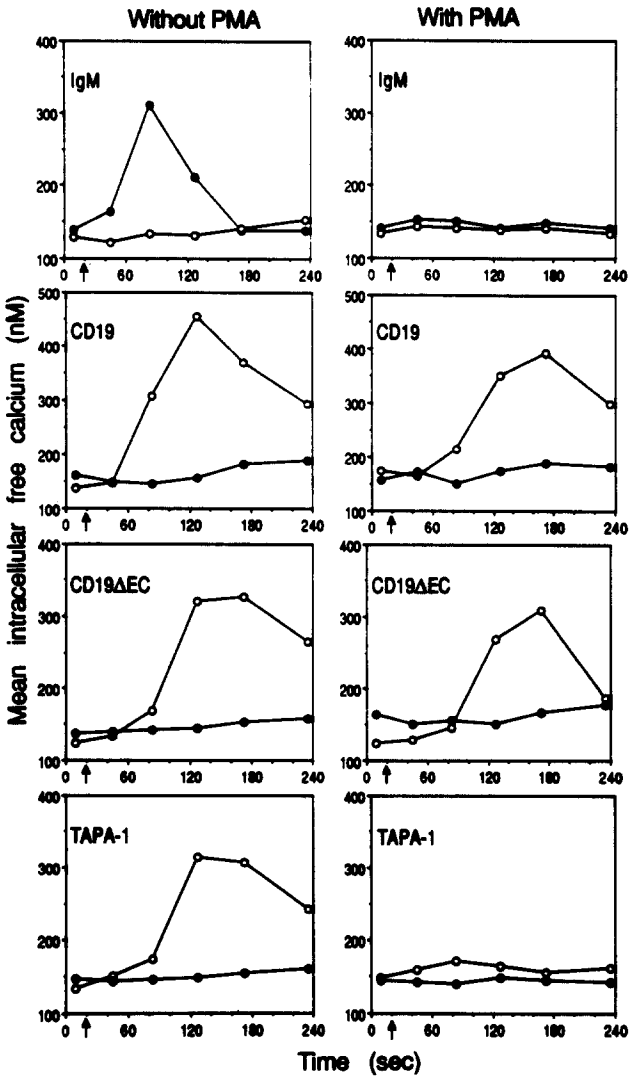

Figure 8. Synergistic increases in $[Ca^{2+}]_i$ in Daudi transfectants induced by cross-linking mIgM, CD19, CD19ΔEc, or TAPA-1 alone or together in the absence or presence of PMA. The Daudi transfectants that had been loaded with indo-1 were preincubated with appropriate mAbs which were cross-linked by the addition (*arrow*) of F(ab')$_2$ goat anti-mouse Ig. Changes in fluorescence of indo-1 were monitored by flow cytometry. The top panels show the response of cells that had been incubated with optimal (2 μg/ml) (●) and suboptimal (0.004 μg/ml) (○) Fab DA4.4 anti-IgM. The other panels depict the results of cells that had been activated by cross-linking antibodies to CD19, to CD4 to ligate CD19ΔEc, and to TAPA-1 either alone (●) or together with suboptimal Fab DA4.4 anti-IgM (○). The concentrations of anti-CD19, anti-CD4, and anti-TAPA-1 mAbs were adjusted to yield equivalent binding of each, and all reactions were carried out in the absence or presence of 75 nM PMA.

or with that of TAPA-1. The function of TAPA-1 could also be assessed independently of the complex by the use of anti-TAPA-1 because the mAb interacts only with TAPA-1 that is free of the complex. In contrast to the increase in $[Ca^{2+}]_i$ seen after cross-linking saturating amounts of anti-IgM, no increase in $[Ca^{2+}]_i$ was induced by addition of F(ab')$_2$ goat anti-mouse Ig to cells bearing either suboptimal amounts of anti-IgM, or to cells bearing equivalent amounts of anti-

CD19, anti-TAPA-1 or anti-CD4 (Fig. 8). Coligating the CD19ΔEc chimera, TAPA-1, or wild-type CD19 to mIgM induced similar synergistic increases in $[Ca^{2+}]_i$ (Fig. 8). In nontransfected Daudi cells, anti-CD4 alone or in combination with suboptimal concentrations of anti-IgM did not alter $[Ca^{2+}]_i$ (data not shown). In the presence of PMA, the synergistic increase in $[Ca^{2+}]_i$ seen after coligating the CD19ΔEc chimera with mIgM was maintained, as was that obtained with the coligation of wild-type CD19 with IgM, as has been previously reported (17) (Fig. 8). PMA abolished the $[Ca^{2+}]_i$ increase seen when TAPA-1 was coligated to mIgM, or when cells were stimulated with saturating concentrations of anti-IgM alone (Fig. 8). Therefore, two components of the CD21/CD19/TAPA-1 complex, CD19 and TAPA-1, can interact with mIgM synergistically to increase $[Ca^{2+}]_i$, but the CD19 component is responsible for the capacity of the complex to induce resistance to inhibition of PMA.

To determine whether other components of the complex are required for mIgM to induce the tyrosine phosphorylation of CD19 and association with the p85 subunit of PI3-kinase, we immunoprecipitated wild-type CD19 and the CD19ΔEc chimera from lysates of transfected Daudi cells that had been treated with buffer or anti-IgM. Selective immunoprecipitation with monoclonal anti-CD19 and anti-CD4 revealed that both forms were tyrosine phosphorylated to a similar extent (Fig. 9 *A*). Anti-CD4 did not recover tyrosine phosphorylated protein from nontransfected Daudi cells. Reprobing the same immunoblot with anti-p85 revealed the coimmunoprecipitation of this subunit of PI3-kinase with both tyrosine-phosphorylated CD19 and the CD19ΔEc chimera (Fig. 9 *B*), indicating that at least Y484 and Y515, which mediate binding to p85 (20), were tyrosine phosphorylated in the chimera.

Homotypic aggregation of Daudi cells expressing the CD19ΔEc chimera was assessed after treatment with incremental concentrations of mAbs to CD19, TAPA-1, and CD4, respectively, in the presence of EDTA, to exclude LFA-1–dependent effects. Replicate samples of cells were stained with fluoresceinated second antibody to quantitate the relative amounts of bound antibodies. Anti-CD19 and anti-TAPA-1 each caused the cells to aggregate in a dose-dependent manner, whereas anti-CD4 had no effect (Fig. 10). Therefore, the capacity of CD19 to induce this cellular response is dependent on its association with other components of the complex, most likely TAPA-1.

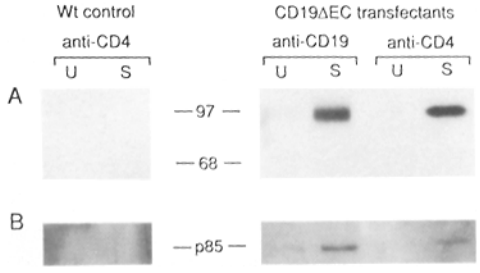

Figure 9. Tyrosine phosphorylation and association of CD19 with the p85 subunit of PI3-kinase. (*A*) Daudi WT and transfectants were stimulated with $F(ab')_2$ goat anti–human IgM (*S*), or treated with buffer alone (*U*). NP-40 lysates were immunoprecipitated with anti-CD4 or anti-CD19, proteins resolved by SDS-PAGE, transferred to nitrocellulose, and probed with ^{125}I-labeled 4G10 antiphosphotyrosine which was detected by autoradiography. (*B*) The immunoblot in (*A*) was reprobed with anti-p85 followed by detection with ECL.

Discussion

A possible biologic role of the CD21/CD19/TAPA-1 complex is to enable the B cell to respond to low concentrations of antigen, despite having low affinity antigen receptors, during the primary immune response by amplifying or altering signal transduction through the antigen receptor. Several biochemical responses mediated by the complex have been described that may contribute to this process: enhanced cellular adhesive properties, augmented activation of PLC, and recruitment of PI3-kinase. Assessing the contribution of each response to the enhancement of B cell activation requires that each be individually elicited or suppressed. To accomplish this task, the complex must be physically and functionally dissected.

10 mutants of CD19, CD21, and CD35 (Fig. 1) were constructed and examined for their capacity to interact with other components of the complex. Substitution of either the transmembrane or extracellular, but not the cytoplasmic, domains of either CD19 (Figs. 2 and 3) or CD21 (Fig. 4) abolished the coimmunoprecipitation of these two proteins, confirming their direct interaction and suggesting that they associate through at least two sites. Precedent for a two-site mode of interaction among membrane protein complexes is the mIgM/Ig-α/β complex in which the transmembrane domains of the heterodimer form a sheath enclosing the conserved polar amino acids of the transmembrane region of mIgM (39), and the ectodomains interact with the CH3 and CH4 regions of mIgM (40). However, CD19 and CD21 differ from the components of the mIgM complex and the TCR complex (41) in lacking many polar or any charged amino acids in their transmembrane domains, and in being able to be expressed independently. Site-directed mutagenesis of the transmembrane domains of these proteins will be necessary to determine if their intramembranous interaction is based on mechanisms similar to those mediating the formation of homo- and heterodimeric complexes by glycophorin A (42) and class II MHC (43) proteins, respectively, membrane proteins with transmembrane domains similarly lacking in polar or charged amino acids. The finding that CD21 could be lost selectively from the complex of CD19 and TAPA-1 (Fig. 2) confirms and extends an earlier finding that CD21 and TAPA-1 did not associate in K562 cells until CD19 was also expressed (1), and does not support a suggestion that CD21 and TAPA-1 interact directly (2). Thus, CD19 is essential for assembly of the CD21/CD19/TAPA-1 complex.

The interaction of CD19 with TAPA-1 required only its extracytoplasmic region (Figs. 2 and 3). We did not alter TAPA-1 to confirm the extracytoplasmic association of these two proteins, but this conclusion would be consistent with the inability of the 5A6 anti-TAPA-1 mAb, whose epitope resides in the second extracellular loop of TAPA-1 (23), to coimmunoprecipitate CD19, if it is assumed that the epitope

is near the site with which CD19 associates. The interaction of CD21 with CD35 was also mediated by the extracytoplasmic regions of these proteins (Figs. 5 and 6) which, if the sites in CD21 for CD19 and CD35 overlap, would explain an earlier demonstration that CD21 did not associate simultaneously with both CD19 and CD35 (38). CD21 must be capable of efficiently capturing immune complexes from CD35 after C3b has been processed to iC3b and C3dg, and after which CD21 must associate with CD19 for the purpose of signal transduction. Mediating the association of CD21 and CD35 through their extracellular domains may be a means by which the binding of ligand regulates this interaction.

There is also precedent in the immune system for interaction among membrane proteins being mediated solely by extracellular domains. Both the IL-2 (44, 45) and IL-6 (46) receptors have ligand binding subunits that associate with signaling subunits through their extracellular regions. The mode of interaction of Leu-13, for which a primary structure has not been defined, is complex, but may involve TAPA-1 and the transmembrane domain of CD19 (Fig. 2). In summary, two of the three interaction domains of the CD21/CD19/TAPA-1 complex are extracytoplasmic, one is intramembraneous, and none is cytoplasmic (Fig. 11).

The objective of these structural studies was to determine rules to guide the construction of a form of CD19 that would not interact with other components of the complex in a B lymphoblastoid cell line (Fig. 7) and which would allow three questions to be posed: (*a*) was CD19 solely responsible for the capacity of the CD21/CD19/TAPA-1 complex to interact synergistically with mIgM for increasing $[Ca^{2+}]_i$; (*b*) did CD19 need to be contained within the complex to be a substrate for a protein tyrosine kinase activated by mIgM; and was the homotypic cellular aggregation induced by antibodies to CD19 (13) dependent on its association with TAPA-1? The CD19ΔEC chimera replicated the capacity of the complex to cause amplified signaling with mIgM that was resistant to inhibition by PMA (Fig. 8). This evidence for CD19 altering in a qualitative as well as quantitative manner the PMA-sensitive signaling process initiated by mIgM had been previously noted when crosslinking the complex to mIgM augmented activation of PLC without increasing the tyrosine phosphorylation of PLCγ, the mechanism of PLC activation by mIgM alone (47). This effect of CD19ΔEC contrasted with that of TAPA-1 which only enhanced the increase in $[Ca^{2+}]_i$ when cross-linked to mIgM, but did not render the response resistant to PMA (Fig. 8). This differential effect may relate to the capacity of anti-CD19, but not anti-TAPA-1, to decrease the threshold of B cell proliferation when coligated with mIgM (6).

The CD19ΔEC chimera was tyrosine phosphorylated and bound PI3-kinase in a manner that was quantitatively similar to wild-type CD19 contained within the complex (Fig. 9). This finding, when coupled with a previous suggestion that the tyrosine kinase activated by the mIg complex is physi-

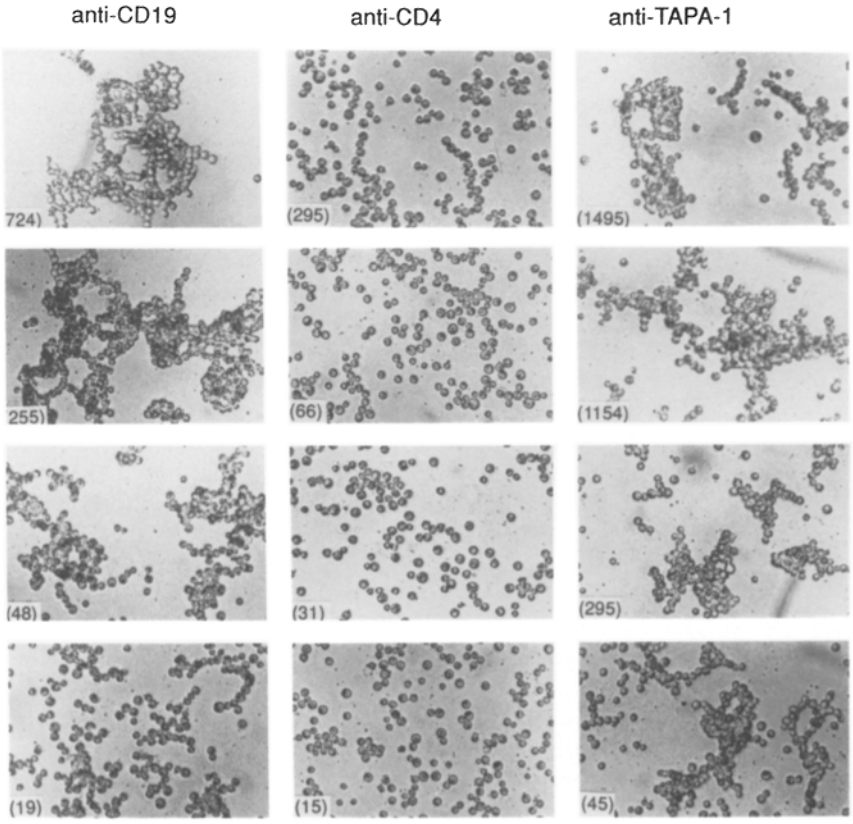

Figure 10. Photomicrographs of transfected Daudi cells that had been incubated with incremental concentrations of anti-CD19 and anti-CD4, which ligate the CD19ΔEc chimera, and anti-TAPA-1, respectively. Replicate samples of cells incubated with identical concentrations of antibody were stained with fluoresceinated goat anti-mouse Ig and analyzed by flow cytometry. The numbers in parentheses represent the mean fluorescent channels of the stained cells.

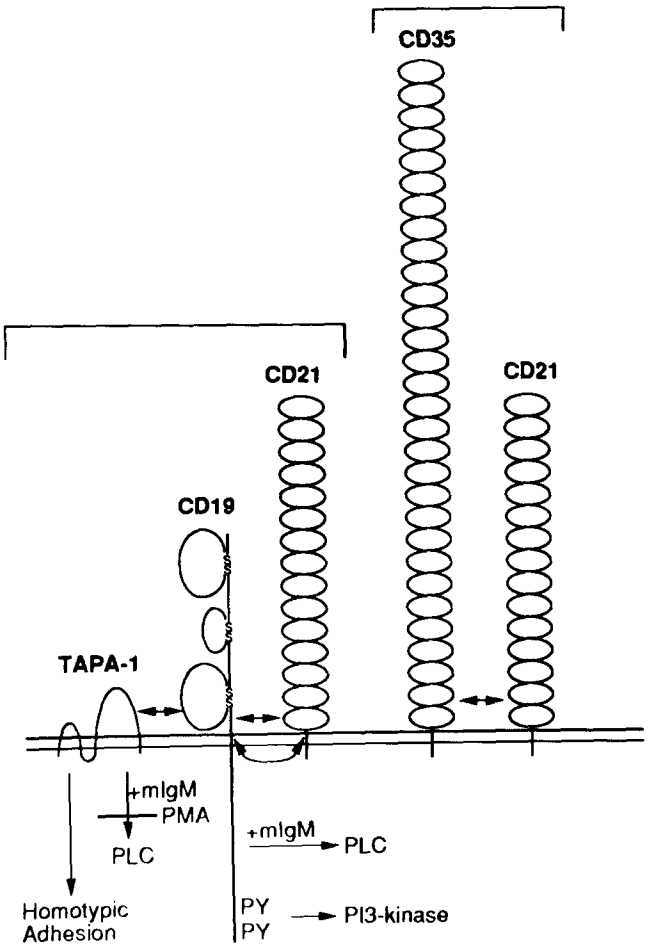

Figure 11. Scheme depicting the domains mediating the interactions of the components of the CD21/CD19/TAPA-1 complex, and the functions of these components.

cally limited to proximate substrates, suggests that the transmembrane or cytoplasmic domain of CD19 is sufficient to localize the protein to the appropriate membrane site for its phosphorylation by the activated tyrosine kinase (48).

The inability of the CD19ΔEC chimera to induce homotypic cellular aggregation (Fig. 10) indicates that the capacity of the CD21/CD19/TAPA-1 complex to cause this response can be ascribed wholly to the TAPA-1 component. This is consistent with earlier observations that anti-TAPA-1 causes the aggregation of hemopoietic cell lines that express TAPA-1 but not CD19 (12), but the possibility that CD19 also had this function could not be excluded until the present study.

The findings of distinct and separable functions of the individual components of the CD21/CD19/TAPA-1 complex lead to a view of the complex as a mosaic comprised of subunits that had evolved independently. Thus, CD19 may have evolved with the immune system, with its specialized functions relating to amplifying signaling through the antigen receptor. TAPA-1 has a general function among hemopoietic cells of promoting cellular adhesion, and may have been recruited to the complex because of the need for B cells to adhere to APCs. CD21 presumably was adapted from other C3-binding proteins to link the recognitive capabilities of the complement system to the earliest phase of the humoral immune response when recognition of antigen is most tenuous.

We wish to thank Dr. Shoshanna Levy for the 5A6 mAb, and Dr. Elizabeth Jaffee for providing the OKT4 mAb.

This work was supported by grants from the National Institutes of Health (5 RO1 AI28191 and 5 RO1 AI22833 to D. T. Fearon, 1 K11 AR01888 to D. R. Martin, and 1 R29 GM 44803 to J. M. Ahearn). A. K. Matsumoto is a Howard Hughes Physician Postdoctoral Fellow, and R. H. Carter and J. M. Ahearn are Arthritis Investigators of the Arthritis Foundation.

Address correspondence to Dr. Douglas T. Fearon, Wellcome Trust Immunology Unit, University of Cambridge School of Clinical Medicine, Hills Road, Cambridge CB2 2SP, UK.

Received for publication 18 June 1993.

References

1. Matsumoto, A.K., J. Kopicky-Burd, R.H. Carter, D.A. Tuveson, T.T. Tedder, and D.T. Fearon. 1991. Interaction of the complement and immune systems: a signal transduction complex of the B lymphocyte containing complement receptor type 2 and CD19. *J. Exp. Med.* 173:55.
2. Bradbury, L.E., G.S. Kansas, S. Levy, R.L. Evans, and T.T. Tedder. 1992. The CD19/CD21 signal transducing complex of human B lymphocytes includes the target of antiproliferative antibody-1 and Leu-13 molecules. *J. Immunol.* 149:2841.
3. Heyman, B., E.J. Wiersma, and T. Kinoshita. 1990. In vivo inhibition of the antibody response by a complement receptor specific monoclonal antibody. *J. Exp. Med.* 172:665.

4. Thyphonitis, G., T. Kinoshita, K. Inoue, J.E. Schweinle, G.C. Tsokos, E.S. Metcalf, F.D. Finkelman, and J.E. Balow. 1991. Modulation of mouse complement receptors 1 and 2 suppresses antibody responses in vivo. *J. Immunol.* 147:224.
5. Hebell, T., J.M. Ahearn, and D.T. Fearon. 1991. Suppression of the immune response by a soluble complement receptor of B lymphocytes. 1991. *Science (Wash. DC).* 254:102.
6. Carter, R.H., and D.T. Fearon. 1992. CD19: lowering the threshold for antigen receptor stimulation of B lymphocytes. *Science (Wash. DC).* 256:105.
7. Carter, R.H., and D.T. Fearon. 1992. Mediating B lymphocyte activation by the CR2/CD19 complex. *In* Progress in Immunology VII. J. Gergely, editor. Springer-Verlag New York Inc., New York. 495.
8. Aubry, J.P., S. Pochon, P. Graber, K.U. Jansen, and J.Y. Bonnefoy. 1992. CD21 is a ligand for CD23 and regulates IgE production. *Nature (Lond.).* 358:505.
9. Tedder, T.T., L.T. Clement, and M.D. Cooper. 1984. Expression of C3d receptors during human B cell differentiation: immunofluorescence analysis with the HB-5 monoclonal antibody. *J. Immunol.* 133:678.
10. Nadler, L.M., K.C. Anderson, G. Marti, M. Bates, E. Park, J.F. Daley, and S.F. Schlossman. 1983. B4, a human B lymphocyte–associated antigen expressed on normal, mitogen-activated, and malignant B lymphocytes. *J. Immunol.* 131:244.
11. Oren, R., S. Takahashi, C. Doss, R. Levy, and S. Levy. 1990. TAPA-1, the target of an antiproliferative antibody, defines a new family of transmembrane proteins. *Mol. Cell. Biol.* 10:4007.
12. Takahashi, S., C. Doss, S. Levy, and R. Levy. 1990. TAPA-1, the target of an antiproliferative antibody is associated on the cell surface with the Leu-13 antigen. *J. Immunol.* 145:2207.
13. Kansas, G.S., and T.T. Tedder. 1991. Transmembrane signals generated through MHC class II, CD19, CD20, CD39, and CD40 antigens induce LFA-1 dependent adhesion in human B cells through a tyrosine kinase–dependent pathway. *J. Immunol.* 147:4094.
14. Carter, R.H., M.O. Spycher, Y.C. Ng, R. Hoffman, and D.T. Fearon. 1988. Synergistic interaction between complement receptor type 2 and membrane IgM on B lymphocytes. *J. Immunol.* 141:457.
15. Tsokos, G.C., J.D. Lambris, F.D. Finkelman, E.D. Anastassiou, and C.H. June. 1990. Monovalent ligands of complement receptor type 2 inhibit whereas polyvalent ligands enhance anti-Ig–induced human B cell intracytoplasmic free calcium concentration. *J. Immunol.* 144:1640.
16. Hivroz, C., E. Fischer, and M.D. Kazatchkine. 1991. Differential effects of the stimulation of complement receptors CR1 (CD35) and CR2 (CD21) on cell proliferation and intracellular Ca^{2+} mobilization of chronic lymphocytic leukemia B cells. *J. Immunol.* 146:1766.
17. Carter, R.H., D.A. Tuveson, D.J. Park, S.G. Rhee, and D.T. Fearon. 1991. The CD19 complex of B lymphocytes. Activation of phospholipase C by a protein tyrosine kinase–dependent pathway that can be enhanced by the membrane IgM complex. *J. Immunol.* 147:3663.
18. Pezzutto, A., B. Dorken, P.S. Rabinovitch, J.A. Ledbetter, G. Moldenhauer, and E.A. Clark. 1987. CD19 monoclonal antibody HD37 inhibits anti-immunoglobulin–induced B cell activation and proliferation. *J. Immunol.* 138:2793.
19. Callard, R.E., K.P. Rigley, S.H. Smith, S. Thurstan, and J.G. Shields. 1992. CD19 regulation of human B cell responses. B cell proliferation and antibody secretion are inhibited or enhanced by ligation of the CD19 surface glycoprotein depending on the stimulation signal used. *J. Immunol.* 148:2983.
20. Tuveson, D.A., R.H. Carter, S.P. Soltoff, and D.T. Fearon. 1993. CD19 of B cells as a surrogate kinase insert region to bind phosphatidylinositol 3-kinase. *Science (Wash. DC).* 260:986.
21. Stamenkovic, I., and B. Seed. 1988. CD19, the earliest differentiation antigen of the B cell lineage, bears three extracellular immunoglobulin-like domains and an Epstein-Barr virus–related cytoplasmic tail. *J. Exp. Med.* 168:1205.
22. Tedder, T.T., and C.M. Isaacs. 1989. Isolation of cDNAs encoding the CD19 antigen of human and mouse B lymphocytes. A new member of the immunoglobulin superfamily. *J. Immunol.* 143:712.
23. Levy, S., V.Q. Nguyen, M.L. Andria, and S. Takahashi. 1991. Structure and membrane topology of TAPA-1. *J. Biol. Chem.* 266:14597.
24. Jaffe, E.A., D. Armellino, G. Lam, C. Cordon-Cardo, H.W. Murray, and R.L. Evans. 1989. IFN-γ and IFN-α induce the expression and synthesis of Leu-13 antigen by cultured human endothelial cells. *J. Immunol.* 143:3961.
25. Pumarola-Sune, T., F. Graus, Y.-X. Chen, C. Cordon-Cardo, and R.L. Evans. 1986. A monoclonal antibody that induces T cell activation reacts with vascular endothelial cells and placental trophoblasts. *J. Immunol.* 137:826.
26. Evans, S.S., D.B. Lee, T. Han, T.B. Tomasi, and R.L. Evans. 1990. Monoclonal antibody to the interferon-inducible protein Leu-13 triggers aggregation and inhibits proliferation of leukemic B cells. *Blood.* 76:2583.
27. Cleavers, H., B. Alarcon, T. Wileman, and C. Terhorst. 1988. The T cell receptor/CD3 complex: a dynamic protein ensemble. *Annu. Rev. Immunol.* 6:629.
28. Reth, M. 1992. Antigen receptors on B lymphocytes. *Annu. Rev. Immunol.* 10:97.
29. Campbell, K.S., and J.C. Cambier. 1990. B lymphocyte antigen receptors (mIg) are non-covalently associated with a disulfide linked inducibly phosphorylated glycoprotein complex. *EMBO (Eur. Mol. Biol. Organ.) J.* 9:441.
30. Lowell, C.A., L.B. Klickstein, R.H. Carter, J.A. Mitchell, D.T. Fearon, and J.M. Ahearn. 1989. Mapping of the Epstein-Barr virus and C3dg binding site to a common domain on complement receptor type 2. *J. Exp. Med.* 170:1931.
31. Caral, J.C., B.L. Myones, B. Frazier, and V.M. Holers. 1990. Structural requirements for C3dg/Epstein-Barr virus receptor (CR2/CD21) ligand binding, internalization, and viral infection. *J. Biol. Chem.* 265:12293.
32. Changelian, P.S., R.M. Jack, L.A. Collinis, and D.T. Fearon. 1985. PMA induces the ligand-independent internalization of CR1 on human neutrophils. *J. Immunol.* 134:1851.
33. Cepko, C., B. Roberts, and R. Mulligan. 1984. Construction and applications of a highly transmissable retrovirus shuttle vector. *Cell.* 37:1053.
34. Martin, D.R., A. Yuryev, K.R. Kalli, D.T. Fearon, and J.M. Ahearn. 1991. Determination of the structural basis for selective binding of Epstein-Barr virus to human complement receptor type 2. *J. Exp. Med.* 174:1299.
35. Miller, A., and G. Rosman. 1989. Improved retroviral vectors for gene transfer and expression. *Biotechniques.* 7:980.
36. Kunkel, T.A., J.D. Roberts, and R.A. Zakour. 1987. Rapid and efficient site specific mutagenesis without phenotypic selection. *Methods Enzymol.* 154:367.
37. Thompson, J.A., A.L. Lau, and D.D. Cunningham. 1987. Selective radiolabeling of cell surface proteins to a high specific activity. *Biochemistry.* 26:743.
38. Tuveson, D.A., J.M. Ahearn, A.K. Matsumoto, and D.T.

Fearon. 1991. Molecular interactions of complement receptors on B lymphocytes: a CR1/CR2 complex distinct from the CR2/CD19 complex. *J. Exp. Med.* 173:1083.
39. Williams, G.T., A.R. Venkitaranan, D.J. Gilmore, and M.S. Neuberger. 1990. The sequence of the μ transmembrane segment determines the tissue specificity of the transport of immunoglobulin M to the cell surface. *J. Exp. Med.* 171:947.
40. Hombach, J., F. Lottspeich, and M. Reth. 1990. Identification of the genes encoding the IgM-α and Ig-β components of the IgM antigen receptor complex by amino-terminal sequencing. *Eur. J. Immunol.* 20:2795.
41. Morley, B.J., K.N. Chin, M.E. Newton, and A. Weiss. 1988. The lysine residue in the membrane spanning domain of the β chain is necessary for cell surface expression of the T cell antigen receptor. *J. Exp. Med.* 168:1971.
42. Lemmon, M.A., J.M. Flanagan, J.F. Hunt, B.D. Adair, B.J. Bormann, C.E. Dempsey, and D.M. Engleman. 1992. Glycophorin A dimerization is driven by specific interactions between transmembrane α-helices. *J. Biol. Chem.* 267:7683.
43. Cosson, P., and J.S. Bonifacino. 1992. Role of transmembrane domain interactions in the assembly of class II MHC molecules. *Science (Wash. DC).* 258:659.
44. Hatakeyama, M., T. Doi, T. Kono, M. Maruyama, S. Minamoto, H. Mori, M. Kobayashi, T. Uchiyama, and T. Taniguchi. 1987. Transmembrane signalling of interleukin 2 receptor: conformation and function of human interleukin 2 receptor (p55)/insulin receptor chimeric molecules. *J. Exp. Med.* 166:362.
45. Kondo, S., M. Kinoshita, M. Shimizu, Y. Saito, M. Kunishi, H. Sabe, and T. Honjo. 1987. Expression and functional characterization of artificial mutants of interleukin-2 receptor. *Nature (Lond.).* 327:64.
46. Taga, T., M. Hibi, Y. Hirata, K. Yamasaki, K. Yasukawa, T. Matsuda, T. Hirano, and T. Kishimoto. 1989. Interleukin-6 triggers the association of its receptor with a possible signal transducer, gp130. *Cell.* 58:573.
47. Carter, R.H., D.J. Park, S.G. Rhee, and D.T. Fearon. 1991. Tyrosine phosphorylation of phospholipase C by membrane IgM in B lymphocytes. *Proc. Natl. Acad. Sci. USA.* 88:2745.
48. Gold, M.R., L. Matsuuchi, R.B. Kelly, and A.L. DeFranco. 1991. Tyrosine phosphorylation of components of the B cell antigen receptor following receptor crosslinking. *Proc. Natl. Acad. Sci. USA.* 88:3436.