CD19 Has a Potential CD77 (Globotriaosyl Ceramide)-binding Site with Sequence Similarity to Verotoxin B-subunits: Implications of Molecular Mimicry for B Cell Adhesion and Enterohemorrhagic *Escherichia coli* Pathogenesis

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Summary

The glycosphingolipid globotriaosyl ceramide (CD77) and other globo-series glycolipids containing terminal galactose (Gal)α1-4Gal residues function as receptors for the verotoxin (Shiga-like toxin) family of *Escherichia coli*-elaborated toxins. CD77 is also a marker for germinal center B lymphocytes and Burkitt's lymphoma cells. The pan B cell marker CD19 is a 95-kD membrane protein that appears early in B cell differentiation and is only lost upon terminal differentiation to plasma cells. CD19 is involved in signal transduction and has a regulatory role in B cell proliferation and differentiation in response to activation in vitro. However, an endogenous ligand for CD19 has not yet been identified. We report herein that the extracellular domain of CD19 has a potential CD77-binding site with extensive sequence similarity to the verotoxin B-subunits. These B-subunit–like sequences on CD19 are in close proximity following the organization of intervening amino acids into disulfide-linked domains. Cocapping of CD19 and CD77 on Burkitt's lymphoma–derived Daudi cells with anti-CD19 antibodies indicates that CD19 and CD77 are associated on the B cell surface. Cell surface binding of anti-CD19 antibodies is decreased on CD77-deficient mutant Daudi cells, suggesting that CD77 expression influences the surface expression of CD19. Wild-type Daudi cells, but not the CD19/CD77-deficient mutants, bind to matrices expressing the carbohydrate moiety of CD77 or other Galα1-4Gal containing glycolipids. This binding can be inhibited by anti-CD77 antibodies, the CD77-binding verotoxin B-subunit or anti-CD19 antibodies. Daudi cells exhibit a degree of spontaneous homotypic adhesion in culture while the CD77/CD19-deficient Daudi mutants grow as single cells. The stronger homotypic adhesion that occurs in B cells after antibody ligation of CD19 and that involves, to some extent, the integrin system, is also dramatically lower in the mutant cells relative to the parent cell line. However, reconstitution of mutant cells with CD77 restores the anti-CD19 mAb-induced adhesion to wild-type Daudi cell levels. These studies represent the first time that CD19-mediated signaling has been reconstituted in a low-responder B cell line. These convergent observations provide compelling evidence that CD19/CD77 interactions function in adhesion and signal transduction at a specific stage in B cell development and suggest that such interactions have a role in B lymphocyte homing and germinal center formation in vivo. By targeting $CD77^+$ B cells, verotoxins may suppress the humoral arm of the immune response during infection. This may explain the infrequent and sporadic nature of antitoxin antibody responses in individuals exposed to verotoxin-producing *E. coli*.

Verotoxins (VTs)[1] produced by serotype 0157:H7 and other enterohemorrhagic *Escherichia coli* have been implicated in the etiology of hemorrhagic colitis and hemolytic uremic syndrome (the so-called "hamburger disease") (1). Also known as Shiga-like toxins (2), they are composed of a single A-subunit with *N*-glycanase activity and a pentameric array

[1] *Abbreviations used in this paper:* ASA, acetylated silylaminated 8-methoxycarbonyloctyl linkage arm; DGDG, digalactosyl diglyceride; FDC, follicular dendritic cells; Gal, galactose; GalNac, *N*-acetyl galactosamine; Gb_4, globotetraosyl ceramide; Glc, glucose; GlcNac, *N*-acetyl glucosamine; TRITC, tetramethylrhodamine isothiocyanate; VT, verotoxin; VTB, verotoxin B-subunit; VT1 B, verotoxin 1 B-subunit.

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of noncovalently associated B-subunits (3, 4). The A-subunit cleaves a specific adenine residue on the 28S ribosomal subunit causing inhibition of protein synthesis and, ultimately, cell death (5, 6). B-subunits specifically target terminal galactose (Gal)α1 → 4 Gal residues of globoseries glycosphingolipids exposed on cell surfaces (7–9). Although terminal Galα1 → 4 Gal residues are present on CD77 (globotriaosyl ceramide [Gb_3]), galabiosyl ceramide, and P1 blood-group glycolipids, CD77 is by far the most common of these glycosphingolipids and is the only one yet shown to function as a receptor for VT on human cells (10). The internal Galα1 → 4 Gal residues of globotetraosyl ceramide (Gb_4–globoside) are not recognized by VTs associated with human disease (11). However, both CD77 and Gb_4 can function as receptors for VT2e (11, 12), which has been implicated in the etiology of edema disease in swine (13). Terminal digalactosyl residues on a diglyceride backbone (9, 14, 15) or as free oligosaccharide (8) are not recognized by VTs. While CD77 is found on a variety of human cell types in vitro, it has been proposed that certain cells, notably pediatric glomerular endothelial cells, are especially susceptible to the effects of VT in vivo (16).

Evidence in support of a specific role for CD77 in B cell differentiation includes reports that CD77 expression in B lymphocytes is largely restricted to germinal center cells (17, 18) and that IgG and IgA responses are selectively inhibited by VT in vitro (19) and, possibly, in vivo (20), whereas IgM responses remain relatively intact. Burkitt's lymphoma cell lines express high levels of CD77 (21) and thus may serve as in vitro models for germinal center B cells. Mutants of the Burkitt's lymphoma–derived Daudi cell line that are deficient in CD77 are resistant to both the cytotoxic effects of verotoxin and the antiproliferative effects of type I IFN (22). Similarities in amino acid sequences (23) between verotoxin B-subunits (VTBs) and the NH_2 terminus of the 63-kD subunit of the human IFN-α receptor (24) suggest a direct role for CD77 in IFN signaling. This has recently been confirmed (25, 26).

A search of the National Biomedical Research Foundation (NBRF) protein data bank for proteins with amino acid sequences similar to those of the VTBs, identified CD19 as another potential CD77-binding protein. CD19, the earliest marker of cells committed to the B lymphocyte lineage, is only lost upon terminal differentiation to plasma cells (27). It forms a complex on the B cell surface with CD21, TAPA-1, and Leu-13 (28, 29). Although an endogenous ligand for CD19 has not been described, antibody ligation of CD19 has been shown to modulate signal transduction, adhesion, proliferation, and differentiation following B cell activation (28, 30–36). CD19 is a 95-kD protein with an extracellular region that includes three potentially disulfide-linked domains, two of which are immunoglobulin-like, a short hydrophobic transmembrane region, and a long cytoplasmic tail with multiple potential phosphorylation sites (37). We propose that a CD77-binding site in the extracellular region of CD19 is involved in the homotypic adhesion and homing of germinal center B cells, and that CD77 is a component of the CD19 membrane complex. By targeting CD77-positive B lymphocytes, VTs may allow enterohemorrhagic *E. coli* to evade the humoral arm of the immune response during infection.

Materials and Methods

Reagents. Verotoxin 1 B-subunit (VT1 B) was isolated as previously described (4). Anti-CD19 (clone B4 and B4-FITC) and isotypic control antibodies were purchased from Coulter Electronics (Hialeah, FL) and tetramethylrhodamine isothiocyanate (TRITC)-conjugated goat anti-mouse IgG from Sigma Chemical Co. (St. Louis, MO). Anti-CD77 mAb (clone 38.13) was a gift of J. Wiels (Institut Gustave Roussy, Villejuif Cedex, France). CD77, P1 and Gb_4-based Synsorb matrices were a gift of G. Armstrong (University of Alberta, Edmonton, Canada). Acetylated silylaminated 8-methoxycarbonyloctyl linkage arm (ASA) and β-glucose matrices were from O. Hindsgaul (University of Alberta).

Immunofluorescence and Flow Cytometry. Daudi cells and the Daudi-derived VT500 mutant were maintained as described (38). VT500 cells were periodically checked for $CD77^+$ revertants by immunofluorescence using VT1 B-subunit conjugated to FITC (VTB-FITC) (16). VTB-FITC and primary antibodies were used at 10 μg/ml unless otherwise indicated. Capping of CD19 was performed by incubating cells with anti-CD19 mAb for 30 min at 4°C, followed by TRITC-conjugated goat anti-mouse IgG (Sigma Chemical Co.) at 37°C for 1.5 h. Cells were then washed in cold PBS and incubated with VTB-FITC for 30 min at 4°C. Capping of CD77 was performed by incubation of cells with VT1 B-FITC at 37°C for 1 h. Cells were then washed in cold PBS and incubated with anti-CD19 followed by TRITC-2nd antibody at 4°C. Double-labeling of VT500 revertant cells for CD77/CD19 was performed by incubating cells (5×10^5) with VTB-FITC and anti-CD19 mAb at 4°C for 30 min. The cells were washed in cold PBS and TRITC-conjugated anti-mouse IgG was added at 4°C for 30 min. For flow cytometry, Daudi and VT500 cells were labeled with B4-FITC or IgG1-FITC (Coulter Electronics) for 30 min at 4°C and washed in cold PBS. Cells were then analyzed on an Epics Profile Analyzer (Coulter Electronics).

Binding of Cells to Glycolipid-based Carbohydrate Matrices. Cells (5×10^5) in 1 ml RPMI 1640 with 10% FBS were added to 250 μg of oligosaccharide matrices (39) and incubated at 37°C, 5% CO_2, for 24 h. The VT1 B and mAbs against CD77 and CD19 were used at 10 μg/ml. For the assays in which Galα1-4Gal–containing matrices were blocked with anti-CD77 or VT1 B-subunit, results shown are for P1 oligosaccharide matrices that had been preincubated with 10 μg of mAb or B-subunit for 2 h in PBS at 4°C. They were then washed in PBS before addition to cells. Daudi cell aggregates were dispersed through a 50-μm mesh cell strainer (Becton Dickinson & Co., Mountain View, CA) before binding assays.

Reconstitution of VT500 Cells with Glycolipids Incorporated into Liposomes and Anti-CD19 Antibody-induced Homotypic Adhesion Assays. CD-77 deficient VT500 cells were reconstituted with CD77, Gb_4, or digalactosyl diglyceride (DGDG) incorporated into fusigenic liposomes at 4×10^6 cells/ml according to previously published methods (10, 38). These cells, along with VT500 and Daudi cell controls at 10^6 cells/ml, were then treated with anti-CD19 antibody at 10 μg/ml and subsequently monitored for increased homotypic adhesion.

Results

CD19 Has a Potential CD77 Binding Site Based on Sequence Similarities to VTBs. The similarities in amino acid compo-

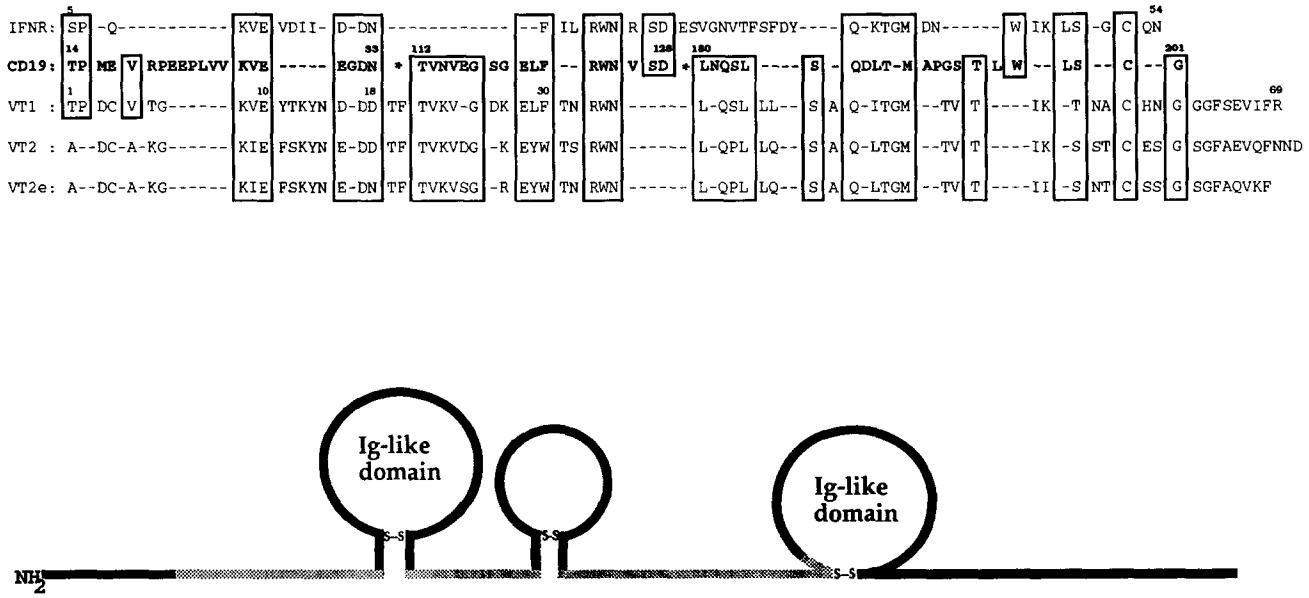


Figure 1. Comparison of amino acid sequences from the extracellular regions of CD19 and the 63-kD subunit of the IFN-α receptor with VTB sequences. (*A*) Asterisks represent intervening sequences in CD19 that do not resemble VTB sequences. Numerical designations for CD19 NH_2-terminal amino acids are from the NBRF protein data bank (41). VTB sequences are given in their entirety. (*B*) Extracellular region of CD19 organized into disulfide-linked domains (37). VT-like sequences are shaded grey.

sition between VTBs and the corresponding sequences in the extracellular domain of CD19 (40, 41) are aligned in Fig. 1 *A*. The similarity is quite extensive and includes several sequences which are not shared with the 63-kD subunit of the IFN-α receptor. A model for CD19 has been proposed in which the intervening, VT-dissimilar sequences form disulfide-linked domains (37), thus bringing the VT-like regions into close proximity (Fig. 1 *B*). When optimally aligned, the VT-like CD19 sequences have 41%, 34%, and 37% identity to VT1, VT2, and VT2e B-subunits, respectively. If these CD19 sequences are compared with a "consensus" sequence of VTBs, then the identity rises to 46% (49% if conservative substitutions are included). The space-filling model of VT1 B (Fig. 2) is based on the crystal structure (42). Most of the amino

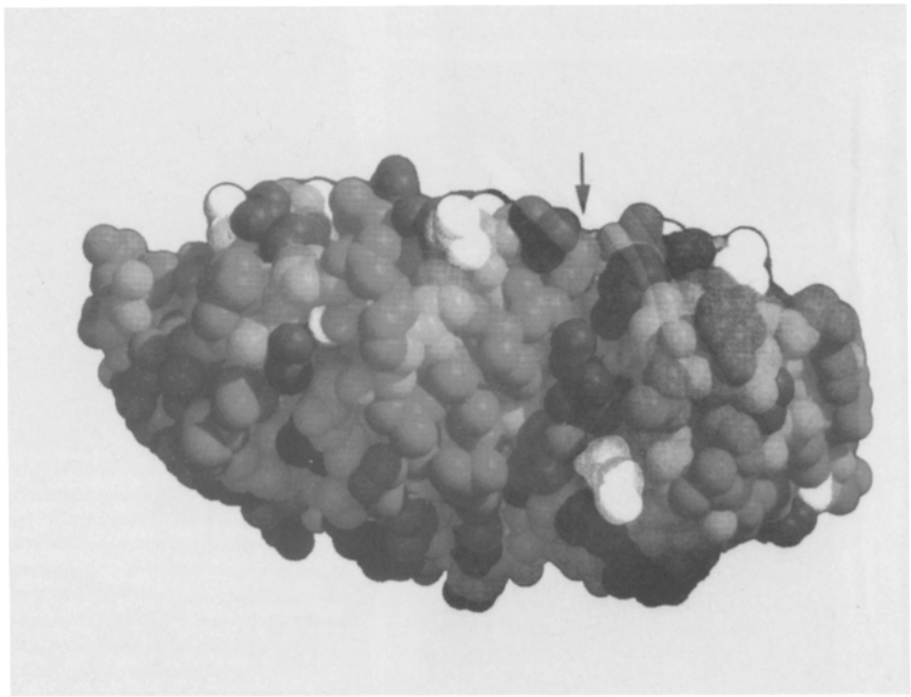


Figure 2. Space-filling model of VT1 B oligomer highlighting amino acids shared with CD19 (as shown in Fig. 1 *A*). Side chains of identical amino acids are shown in black whereas those of similar amino acids are in white. The arrow indicates the proposed CD77-binding cleft (42).

acids shared by the CD19 extracellular region lie within the proposed CD77-binding cleft between monomers.

CD19/CD77 Interactions on B Cells In Vitro. Daudi cells normally express high levels of CD77 (21, 43). Cocapping of CD19 and CD77 with anti-CD19 antibodies indicates that they are associated on the Daudi cell surface (Fig. 3, *A* and *B*). However, the capping of CD77 by the VT1 B does not result in the cocapping of CD19 (Fig. 3, *C* and *D*). Anti-μ mAb also cocaps CD77 (results not shown), which could be due to the presence of CD19 in the antigen receptor complex (30).

Daudi cell mutants that lack CD77 have reduced surface CD19 levels as determined by flow cytometry (Fig. 4 *E*). In contrast, surface levels of CD10, CD20, HLA-DR, and IgM on the VT500 cells are similar to those of wild-type Daudi cells (results not shown). Occasionally, some VT500's will spontaneously revert to the wild-type $CD77^+$ phenotype in culture. These revertants show a coincident increase in CD19 surface expression to a level approximating that of wild-type Daudi cells (Fig. 4, *C* and *D*).

Binding of B Cell Lines to Glycolipid-based Oligosaccharide Matrices. Verotoxins will bind matrices of CD77 oligosac-

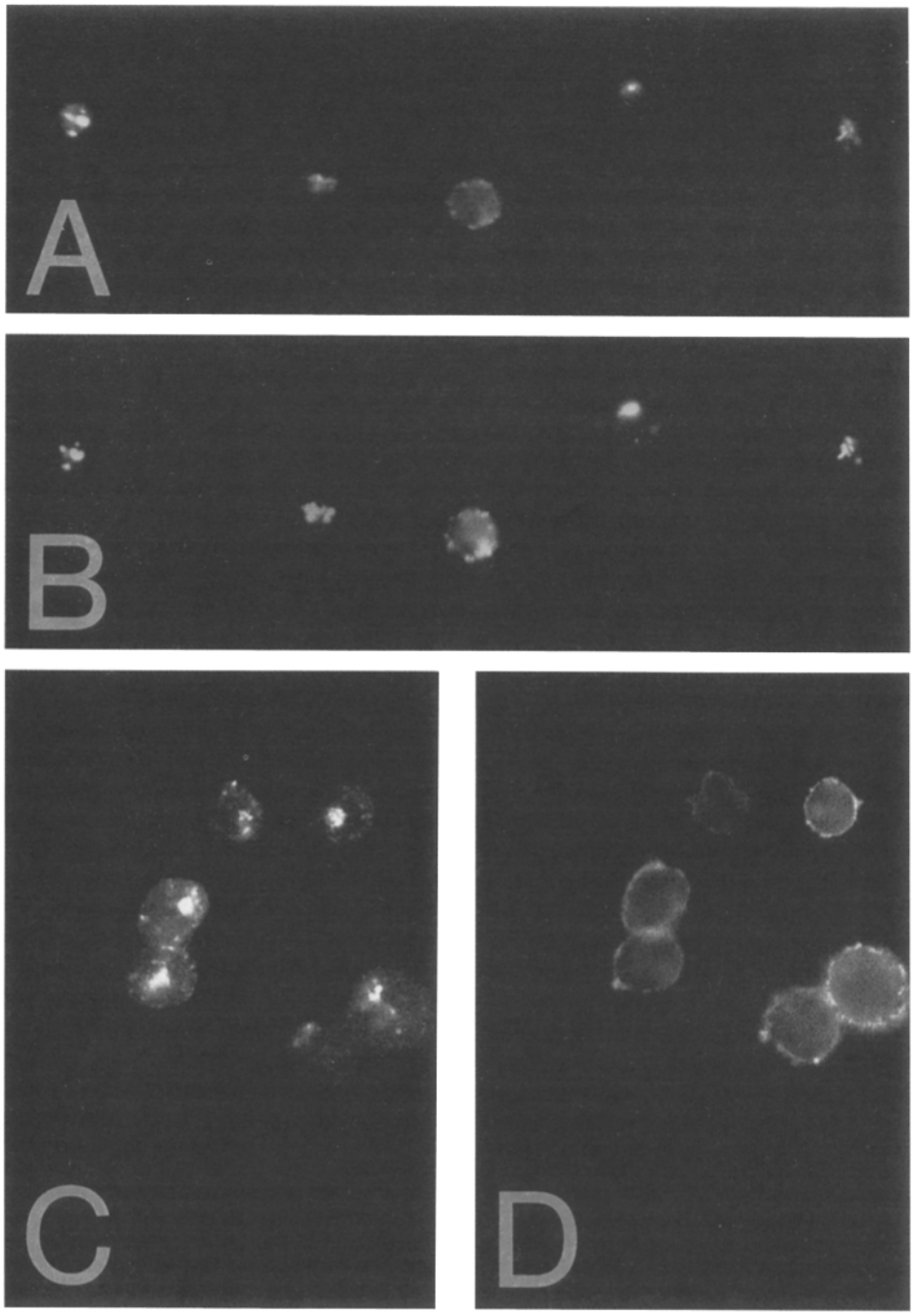


Figure 3. Association of CD19 and CD77 on the surface of Daudi cells. (*A*) Capping of CD19 on wild-type Daudi cells with anti-CD19 mAb and TRITC-conjugated goat anti-mouse IgG results in (*B*) the cocapping of CD77 as visualized by the binding of FITC-conjugated VT1 B (VTB-FITC). (*C*) Capping of CD77 on wild-type Daudi cells with VTB-FITC does not result in the cocapping of CD19 molecules (*D*).

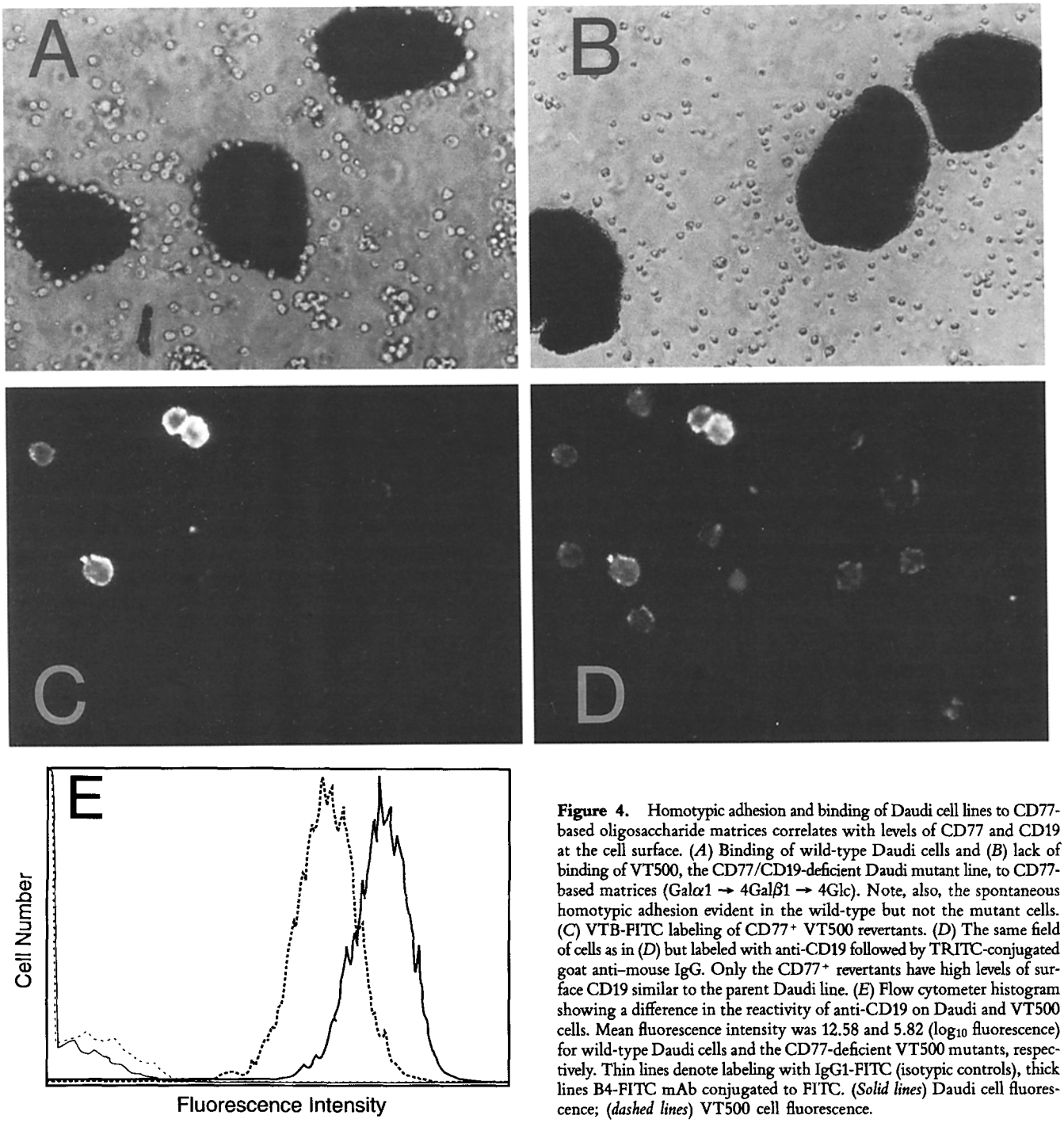
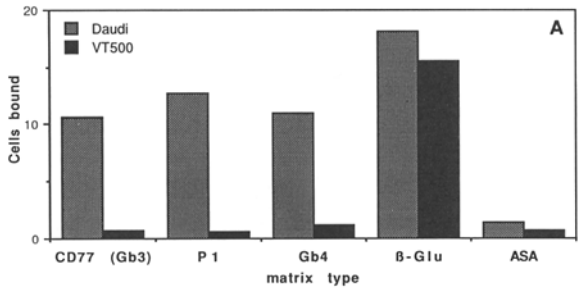
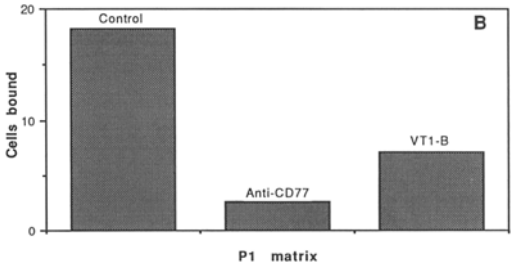


Figure 4. Homotypic adhesion and binding of Daudi cell lines to CD77-based oligosaccharide matrices correlates with levels of CD77 and CD19 at the cell surface. (*A*) Binding of wild-type Daudi cells and (*B*) lack of binding of VT500, the CD77/CD19-deficient Daudi mutant line, to CD77-based matrices (Galα1 → 4Galβ1 → 4Glc). Note, also, the spontaneous homotypic adhesion evident in the wild-type but not the mutant cells. (*C*) VTB-FITC labeling of CD77$^+$ VT500 revertants. (*D*) The same field of cells as in (*D*) but labeled with anti-CD19 followed by TRITC-conjugated goat anti-mouse IgG. Only the CD77$^+$ revertants have high levels of surface CD19 similar to the parent Daudi line. (*E*) Flow cytometer histogram showing a difference in the reactivity of anti-CD19 on Daudi and VT500 cells. Mean fluorescence intensity was 12.58 and 5.82 ($\log_{10}$ fluorescence) for wild-type Daudi cells and the CD77-deficient VT500 mutants, respectively. Thin lines denote labeling with IgG1-FITC (isotypic controls), thick lines B4-FITC mAb conjugated to FITC. (*Solid lines*) Daudi cell fluorescence; (*dashed lines*) VT500 cell fluorescence.

charides (Galα1-4Galβ1-4 glucose [Glc]) and other terminal Galα1 → 4Gal matrices (39). Wild-type Daudi cells, but not the CD19-deficient VT500 cells, also bind Galα1 → 4Gal containing matrices, further implicating CD19 as a mediator of Galα1 → 4Gal adhesion (Fig. 5 *A*). Preincubation of these matrices with anti-CD77 mAb prevents the binding to blood group P1 (Galα1 → 4Galβ1 → 4*N*-acetyl glucosamine [GlcNac]) and CD77-based matrices. The VT1 B inhibits binding to a lesser extent (Fig. 5 *B*), likely due to the pentameric nature of VTBs in aqueous solution that may allow some binding to Galα1 → 4Gal residues on cells and matrices simultaneously, potentially cross-linking cells to the matrices. The VT1 B does not bind to Gb_4 carbohydrate matrices (*N*-acetyl galactosamine [GalNacβ1] → 3Galα1 → 4Galβ1 → 4Glc) (G. Armstrong, personal communication) which contain non-terminal Galα1 → 4Gal residues and there-





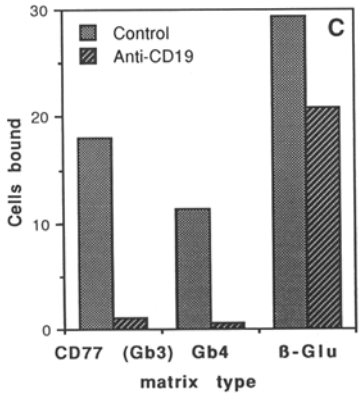


Figure 5. Binding of Daudi cells to Galα1 → 4Gal residues correlates with the availability of CD19 on the cell membrane. (*A*) Binding assays of wild-type Daudi cells and CD77/CD19-deficient VT500 cells to CD77, P1 (Galα1 → 4Galβ1 → 4GlcNac) and Gb_4 or globotetraosyl ceramide (GalNacβ1 → 3Galα1 → 4Galβ1 → 4Glc)-based matrices. β-Glucose and ASA matrices included as controls. The CD19-deficient cells show no significant binding to Galα1 → 4Gal matrices. (*B*) Blocking of Daudi cell binding to Galα1 → 4Gal-containing matrices by anti-CD77 mAb or VT1 B. (*C*) Blocking of Daudi cell binding to Galα1 → 4Gal-containing matrices by anti-CD19 mAb. Anti-CD19 mAb was added to Daudi cells just prior to their addition to CD77, Gb_4, or β-glucose (control)-based carbohydrate matrices.

fore does not inhibit Daudi cell-Gb_4 matrix adherence. Anti-CD19 antibodies inhibit binding of Daudi cells to all Galα1 → 4Gal containing matrices but not to β-glucose matrices (Fig. 5 *C*). The CD19-deficient Daudi mutants are also capable of β-glucose binding (Fig. 5 *A*).

CD19/CD77 Mediated Homotypic Adhesion. In contrast to Daudi cells that spontaneously exhibit homotypic adhesion in culture, the CD77/CD19-deficient Daudi mutants grow as single cells (Fig. 4, *A* and *B*). The stronger adhesion, which is induced in Daudi cells after ligation of CD19 by antibody, is also dramatically reduced in VT500 cells (Fig. 6, *A* and *B*). However, levels of antibody-induced adhesion similar to that observed in Daudi cells can be induced in VT500 cells that have been reconstituted with CD77 but not Gb_4, DGDG, or liposome phospholipids, alone (Fig. 6, *C–F*). Results shown are cells at 40 h post-treatment with anti-CD19 antibody.

Discussion

The binding specificity of VT B-subunits for Galα1 → 4Gal residues of glycosphingolipids has been extensively investigated (7–9, 12, 14, 15, 44, 45). Reconstitution of VT-resistant cells with CD77 (10) or Gb_4 (38) and the induction of CD77 synthesis (46, 47) have shown that these globoseries glycolipids are functional cell surface receptors for VTs. By targeting globoseries glycolipids on human cells, VTs provide important tools for investigating the function of these glycosphingolipids in normal cell physiology.

Recently it has been shown that VTBs resemble the extracellular domain of the 63-kD subunit of the IFN-α receptor in their amino acid sequences (23). This provided a molecular explanation for the earlier observation that cells that were CD77-deficient were resistant to both IFN-α–induced growth inhibition and VT cytotoxicity (22, 48). Similarly, a role for CD77 in B cell development was anticipated due to the restricted appearance of CD77 in human B cell stages (18, 49, 50) and the differential action of VT on B cell populations (18, 19, 51).

The VT-like amino acid sequence of the IFN-α receptor subunit is contiguous and lies near the NH_2 terminus in the extracellular domain (23). The majority of these shared amino acids are also found in CD19. However, additional VT-like sequences are present in CD19 that are not in the IFN-α receptor subunit (Fig. 1). Although the VT-like sequences of the CD19 extracellular region are not contiguous, the predicted secondary structure of CD19 results in their close apposition. A common structural motif for carbohydrate-binding proteins, including the VTB, has been described in which a barrel of antiparallel β-sheets is capped by a α-helix (52). The CD19 sequences which resemble VTBs include the B-subunit α-helix (residues 180–189 of CD19), whereas the corresponding IFN-α receptor subunit sequences do not. However, the amino acids of the α-helix do not appear to be directly involved in carbohydrate-binding (42). VTB amino acids important for the specific binding of CD77 and Gb_4 glycolipid receptors have been identified using a combination of site-directed mutagenesis, chemical modification, and crystal structure analysis (42, 44, 53, 54). The proposed CD77-binding clefts lie between the monomers of the pentameric B-subunits, suggesting that CD19 oligomers may be required in order to bind CD77. Many of the amino acids shared by

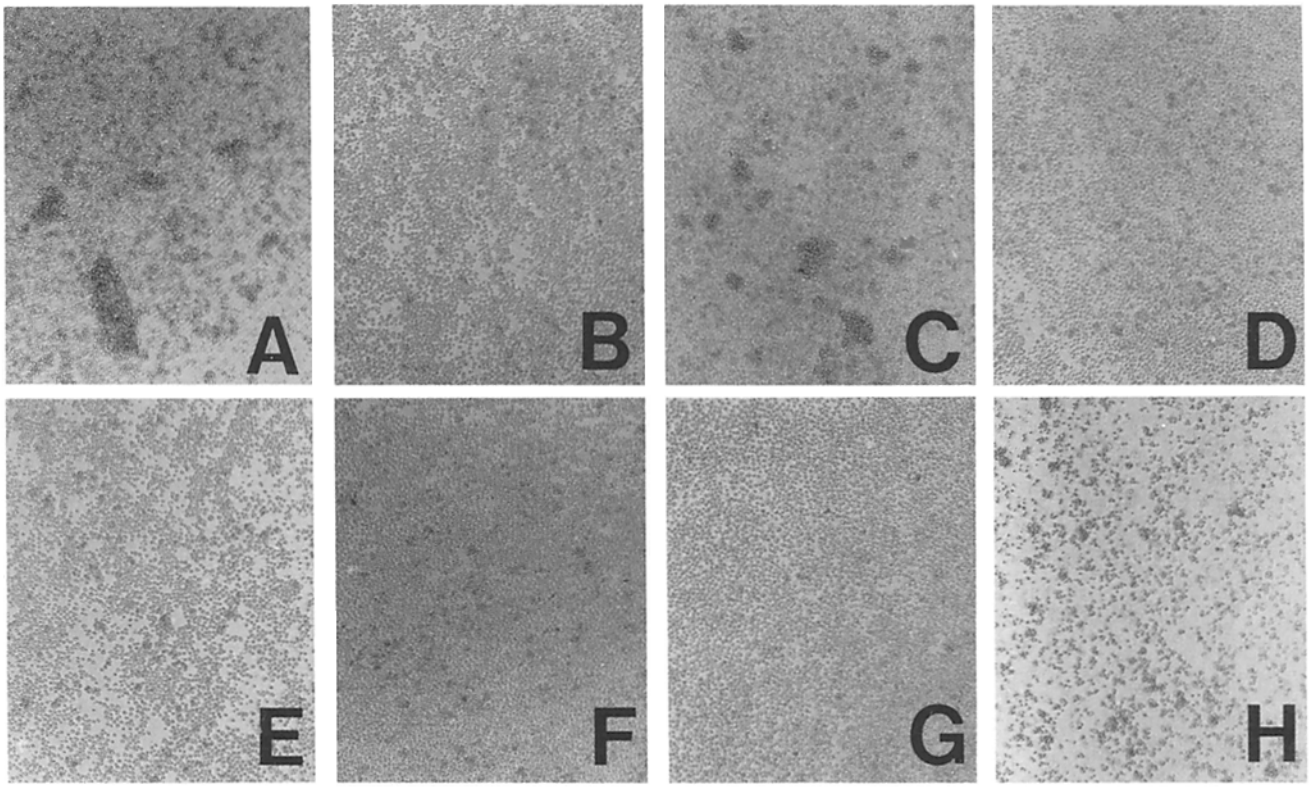


Figure 6. Restoration of anti-CD19 induced homotypic adhesion in VT500 cells reconstituted with CD77. Homotypic adhesion after antibody ligation of CD19 in (*A*) wild-type Daudi cells; (*B*) VT500 cells; and VT500 cells reconstituted with liposomes containing (*C*) CD77, (*D*) Gb_4, (*E*) DGDG, and (*F*) liposome phospholipids, only. Cells treated with mouse IgG1 as isotypic control: (G) VT500 cells reconstituted with CD77 liposomes (VT500 cells reconstituted with other glycolipid/liposome preparations and normal VT500 cells similar); (*H*) wild-type Daudi cells. IgG1 results are similar to untreated cells in culture (not shown).

CD19 and VTB lie within these clefts (42) (Fig. 2). However, most of the identical amino acids lie in the (n+1) VT B monomer forming the binding cleft (Fig. 2). It may be this that "side" of the cleft is sufficient to define the glycolipid binding specificity and that mutations made in the (n) monomer (53, 55) only compromise carbohydrate access. The shared amino acids include glutamic acids 27 (10; numbers in brackets indicate the corresponding positions of VT1 B amino acids), 30 [16] and 120 [28] and aspartic acid 32 [17] that have the potential to form hydrogen bonds with polar groups on CD77, and phenylalanine 122 [30] and tryptophan 124 [34] which could stack against a saccharide ring by hydrophobic interactions. In the area of amino acids 30–33 of CD19 [16–18], the EGDN sequence of CD19 most closely resembles the corresponding EDN sequence of VT2e (Fig. 1 *A*), the only member of the VT family that binds internal as well as terminal Galα1 → 4Gal residues of glycolipids (12, 14). Alteration of the VT1 B-subunit using site-directed mutagenesis to change aspartic acid 18 to asparagine, as occurs in VT2e, resulted in a toxin which bound Gb_4 in addition to CD77 (44). The possibility that CD19 may bind both CD77 and Gb_4 is supported by the results of the oligosaccharide matrix binding assays.

Daudi cells bind matrices containing both terminal (CD77 and P1-based) and internal (Gb_4-based) Galα1 → 4Gal residues (Figs. 4 and 5). This binding can be blocked by preincubation of the CD77 and P1 matrices with Galα1 → 4Gal binding agents such as VT1 B or anti-CD77 mAb (Fig. 5 *B*). Treatment of Daudi cells with anti-CD77 mAb and VTB directly was avoided as ligation of surface CD77 has been shown to induce apoptosis in Burkitt's lymphoma cells (51).

Treatment of Daudi cells with anti-CD19 mAb also blocks the binding to Galα1 → 4Gal–containing matrices. Anti-CD19 does not prevent binding of Daudi cells to β-glucose matrices, thus specifically implicating CD19 as a mediator of Galα1 → 4Gal adhesion (Fig. 5 *C*). The CD77-deficient mutant, VT500, has low surface expression of CD19 (Fig. 4) although surface levels of CD10, CD20, HLA-DR, and IgM on the VT500 cells are similar to those of wild-type Daudi cells. VT500 cells do not bind any of the Galα1 → 4Gal matrices although they are capable of β-glucose binding (Fig. 5 *A*). Therefore, the mechanism for β-glucose binding, which is not known, does not involve CD19. VT500 cells also do not exhibit the spontaneous homotypic adhesion typical of Daudi cells in culture, as would be expected if such interactions are mediated through the binding of CD19 to CD77 molecules on adjacent cells in a manner analogous to Daudi cell CD19/matrix CD77 binding. VT500 cells that have

reverted to express wild-type levels of CD77 also express wild-type CD19 levels. It is possible that the presence of CD77 changes the conformation of CD19, thereby causing an increase in antibody recognition. CD77 binding could direct the folding of nascent CD19 protein such that a CD77-binding site, stabilized by disulfide-linked domains (37), is formed (Fig. 1 *B*). Thus CD19 synthesized in CD77-negative or deficient B cells may lack CD77-binding capability.

The cocapping of CD77 and CD19 by anti-CD19 mAb indicates that they are able to associate on the B cell surface (Fig. 3). Therefore, CD77 could be considered a component of the CD19 complex in Burkitt's lymphoma and germinal center B cells. While CD21 and TAPA-1 are associated with CD19 in this complex, neither possesses VTB-like sequences. Therefore, they are unlikely to be responsible for the cocapping of CD77 and CD19 by anti-CD19 mAb. In contrast, CD19 is not capped by VT1 B. Relative to glycolipids such as CD77, CD19 may be more firmly anchored in the membrane by association with other membrane proteins or cytoskeletal components. However, such "nonreciprocal" cocapping has been previously reported for CD19-associated proteins. For example, anti-IgM caps both surface IgM and CD19 on B cells although anti-CD19 does not cap IgM (30).

The relatively weak "spontaneous" homotypic adhesion of Daudi cells in culture cannot be inhibited by anti-CD19 mAb because antibody ligation of CD19 induces a much stronger homotypic adhesion in B cells. This strong adhesion, which is mediated, in part, through the integrin system (56), may require the interaction of CD19 and TAPA-1, the tetraspan protein member of the CD19 complex (29, 31). This anti-CD19–induced homotypic adhesion is also reduced in the VT500 cells relative to wild-type Daudi cells (Fig. 6 *A* and *B*). Restoration of anti-CD19 induced homotypic adhesion to wild-type levels by reconstitution of VT500 cells with CD77-containing liposomes provides further evidence for a functional interaction between CD19 and CD77 within a cell (Fig. 6, *C–H*). Failure of Gb_4 to reconstitute this strong adhesion mechanism, even though Daudi cells bind both Gb_4- and CD77-based matrices, implicates CD77 as having a specific function in the CD19 signaling pathway. VT that has bound cell surface CD77 undergoes an unusual "retrograde transport" through the Golgi apparatus and endoplasmic reticulum to the nuclear membrane (57). Perhaps CD19-mediated signal transduction requires a retrograde transport mechanism that is provided by CD77, but not Gb_4. Alternatively, CD77 could be modulating this adhesion by regulating CD19 surface expression as discussed above.

Taken together, these observations provide compelling evidence that CD19/CD77 interaction is involved in homotypic adhesion of Daudi cells at two levels: First, our results indicate that the spontaneous homotypic adhesion of Daudi cells in culture is mediated, to some extent, through CD19/CD77 interactions between adjacent $CD77^+CD19^+$ cells. CD19 contains a potential CD77-binding domain similar to that of VTBs, whose ability to bind CD77 and other Galα1 → 4Gal containing glycolipids is well characterized. The Daudi-derived VT500 cells, which are CD77/CD19-deficient relative to the parent cell line, do not exhibit this spontaneous homotypic adhesion and do not bind CD77 or other Galα1 → 4Gal-containing oligosaccharide matrices. Wild-type Daudi cells do bind Galα1 → 4Gal-containing matrices, and this binding can be inhibited by anti-CD19 and anti-CD77 mAb and the VT1 B. Second, our evidence indicates CD77 is involved in the anti-CD19–induced homotypic adhesion which is not mediated directly through CD19/CD77 interaction, but rather through the integrin system and, perhaps, other strong adhesion mechanisms. We propose that CD77's role in this type of homotypic adhesion is due to interaction with CD19 molecules within the same cell. Cocapping of CD19 and CD77 with anti-CD19 mAb indicates that they are associated in the plasma membrane of Daudi cells. The CD77-deficient VT500 cells have reduced surface expression of CD19 as determined by immunofluorescence and flow cytometry. However, VT500 cells that have reverted to express wild-type levels of CD77 also exhibit wild-type CD19 surface expression. CD77 binding could influence the anti-CD19 antibody recognition by inducing a conformational change in the VT-like region of CD19 by directing formation of disulfide-linked extracellular domains. Antibody ligation of CD19 induces dramatically reduced levels of homotypic adhesion in VT500 cells relative to wild-type Daudi cells. Reconstitution of VT500 cells with CD77, but not Gb_4, DGDG, or liposomes alone, restores the anti-CD19 induced homotypic adhesion in these cells to wild-type Daudi cell levels. This provides direct evidence of a functional role for CD77 in the CD19 signaling pathway.

This two-step model of CD19/CD77-mediated adhesion has important implications for B cell adhesion in vivo. Daudi cells and other Burkitt's lymphoma cells are phenotypically similar to a subset of normal germinal center B cells ($CD77^+$ $CD19^+CD10^+CD20^+HLA\text{-}DR^+$) (49). Therefore, CD19/CD77 interactions could be involved in germinal center formation. Follicular dendritic cells (FDCs) are the only cells other than B lymphocytes that express CD19 (58). Because FDCs as well as macrophages in histological sections of lymphoid tissue also have been reported to be $CD77^+$ (59), these cells have the potential to anchor B cells in follicles through CD19/CD77 interactions. Homotypic adhesion of B cells in the germinal centers could then occur, at least initially, through CD19/CD77 binding between adjacent cells in a manner similar to the spontaneous Daudi cell aggregation in culture. Stronger mechanisms of adhesion that could play a subsequent role in the interactions of these cells include the transient LFA-1/intercellular adhesion molecule 1 (ICAM-1) interactions and the other, as yet unidentified, mechanisms of adhesion that occur after antibody ligation of CD19 and other B cell membrane proteins (56, 60). Interestingly, IFN-α also induces a strong homotypic adhesion in B cells through as yet undetermined mechanisms (61). CD77 could play a similar role in this adhesion, as well, through interaction with the extracellular domain of the IFN-α receptor subunit (23). Subsequent downregulation or loss of CD77 from germinal center B cells, perhaps followed by downregulation of surface CD19 expression, would lead to the dispersal

of cells from germinal centers. In support of this model, B cell stages associated with other areas of lymphoid tissue are CD77-negative (62) and the terminally differentiated plasma cells are CD19-negative (27).

The ability of B cells to bind Galα1 → 4Gal residues may also have a function in B lymphocyte homing to sites of inflammation. For example, TNF-α has been shown to upregulate CD77 expression on human endothelial cells (47, 63, 64), and endothelial Gb_4 expression is modulated by IFN-γ (65).

The two step CD19/Galα1 → 4Gal adhesion system that we propose appears to function in a manner analogous to the selectin/sialyl Lewis^x system that mediates the initial "leukocyte rolling" along endothelial cells, with subsequent tighter adhesion mediated by integrins and other adhesion mechanisms (66). However, in this system, the protein (CD19) and glycoconjugate (CD77) appear to function within the cells in the subsequent stronger adhesion mechanisms, as well. Also, P- and E-selectins bind sialyl Lewis^x determinants on both glycolipids and glycoproteins. In contrast, Galα1 → 4Gal determinants on human cells are restricted to glycosphingolipids (26). Therefore, CD77, and possibly Gb_4, would play a central role as endogenous ligands for CD19 in this adhesion system.

These results have important implications for enterohemorrhagic *E. coli* pathogenesis. Antibody responses to VTs are highly variable with the majority of patients exposed to VT never developing an antitoxin titer (20) (67). In vitro, VT causes a significant reduction in antibody production by human tonsillar B cells, with IgG and IgA-producing cells more severely affected than IgM producers (19). CD77$^+$ germinal center B cells have been shown to be highly susceptible to death by apoptosis (18) and CD77$^+$ Burkitt's lymphoma cells die by apoptosis following treatment with VT1 holotoxin or VT1 B alone (51). This inherent sensitivity to apoptosis may be involved in selecting against B cells expressing low affinity antibodies or antibodies that are not specific for epitopes of an invading pathogen. By targeting this population of B cells during infection, VT producing *E. coli* may inhibit maturation of antibody responses including memory cell generation, affinity maturation, and isotype-switching. Antigen presentation may also be impaired as many potential antigen-presenting cells including surface immunoglobulin-positive B cells, FDCs, and certain cells of the myelomonocytic series are CD77$^+$ and thus possible targets of VTs (26). While the cytotoxic activity of VTs on CD77$^+$ cells would result in the modulation of humoral immune responses in general, responses specifically directed against VTs could also be suppressed in order to prevent autoimmune reactions against epitopes shared with CD19 and the 63-kD component of the IFN-α receptor.

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