Brief Definitive Report

CD19 IS FUNCTIONALLY AND PHYSICALLY ASSOCIATED WITH SURFACE IMMUNOGLOBULIN

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The highly conserved transmembrane sequences of surface Igs (sIgs) suggest that these molecules may associate with other integral membrane proteins to execute their dual functions of ligand binding and signal transduction (1, 2). T cell studies suggest that such lineage-specific functions are most likely mediated by lineage-specific antigens (3–5). While four additional B cell–specific surface antigens have been identified (CD19, CD20, CD22, and CD72) (6–9), their functions are unknown.

Since sIg is one of ~30% of B cell surface antigens that modulate, we used a comodulation assay to search for sIg-associated B cell antigens. Antibody-induced antigenic modulation is the rapid, specific, and reversible loss of surface antigens caused by incubating cells at 37°C with an excess of specific antibody (10). Occasionally, chemically distinct antigens comodulate, indicating that they are physically associated. For example, anti-CD3 mAbs comodulate the TCR (T_i) (3, 5). Similarly, modulation of CD3-T_i by antigen or specific antibody comodulates CD4/CD8 (11, 12), confirming the close physical and functional association between CD3-T_i and CD4/CD8 (4). Our studies indicate that the B cell–specific and pan-B sIg and CD19 surface antigens are functionally and physically associated.

Materials and Methods

Cells. Cells were cultured in RPMI 1640 medium (Gibco Laboratories, Grand Island, NY) supplemented with 10% FCS (HyClone Laboratories, Logan, UT). B cells from tonsils of patients at Children's Hospital Medical Center, Seattle, WA, were harvested using standard methods.

mAbs. HB43 (1410KG7), HB57 (DA4-4), and HB45 (141PF11) mAbs specific for Ig γ, μ, and κ chains were obtained from the American Type Culture Collection (Bethesda, MD). Except for fluoresceinated CD19-specific B4 mAb (6) (Coulter Immunology, Hialeah, FL) and CD22-specific G28-7 mAb (Dr. Jeffrey Ledbetter, Oncogen, Seattle, WA), all mAbs were prepared in our own laboratory unless indicated.

mAbs were purified using protein A-Sepharose columns (Sigma Chemical Co., St. Louis, MO), tested by SDS-PAGE, and fluoresceinated using standard methods. Molar fluorescein protein ratios ranged from 6 to 10. Antibody binding was determined using either directly fluoresceinated mAbs or by using fluoresceinated goat anti-mouse antibody (Tago Inc., Burlingame, CA) in indirect immunofluorescence assays monitored on the FACS (Becton Dickinson & Co., Mountain View, CA), with fluorescence displayed in logarithmic mode. All procedures were performed using an excess of mAbs with 0.1% NaN_3 at 4°C to prevent antigenic modulation.

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Modulation. Cells (10^6/ml) are incubated under standard tissue culture conditions with 10 μg/ml of purified mAb for the times indicated (usually 20 h) (10). Identical cultures are incubated with isotype-matched mAbs to sIg, CD19, or CD4 antigens. Changes in binding of directly fluoresceinated mAbs are expressed by dividing the mean channel of fluorescence observed on reactive antibody-treated cells (anti-Ig or anti-CD19) by that observed with control (anti-CD4)-treated cells after correcting for nonspecific binding.

Cocapping. Cells are incubated with directly fluoresceinated mAbs to test antigens and with Texas red-conjugated polyclonal anti-human λ chain-specific antiserum (G/HIg-TR) (Tago Inc.) or unlabeled γ chain-specific mAb (HB43) for 70 min at 37°C, washed, and analyzed using a laser scanning confocal microscope (MRC-500; Bio-Rad Laboratories, Cambridge, MA) with a Nikon Optiphot (Nikon, Torrance, CA). G/HIg-TR does not crossreact with mouse Ig.

Results

Incubation of B cells with an excess of Ig-specific mAbs at 37°C causes rapid, virtually complete loss of sIg (13). Modulation with anti-Ig mAb also selectively reduces binding of fluoresceinated mAbs specific for CD19 (Fig. 1). Binding of J3-119 mAb is reduced on average to 35% of its original value on SKLY18, 34% on Ramos, and 43% on BALM-3 cells. $F(ab)'_2$ fragments of anti-Ig mAbs also comodulate CD19. The process is reversible, since modulated cells incubated without anti-Ig mAbs for 48 h show normal levels of sIg and CD19.

The extracellular region of CD19 contains three Ig-like domains (14). Since sIg and CD19 modulate with anti-Ig and anti-CD19 mAb, respectively (13, 15), comodulation of CD19 by anti-Ig antibodies could reflect serologic crossreactivity between sIg and CD19, but it does not. Comodulation of CD19 is observed when sIgG on SKLY18 cells is modulated by γ or λ chain-specific mAb, when sIgG on BALM-3 cells is modulated by γ or κ chain-specific mAb, and when sIgM on Ramos cells is modulated by μ or λ chain-specific mAb. It is improbable that antibodies specific for sIg γ, μ, κ, and λ chains all crossreact with CD19. Modulation of sIgM on Ramos cells by an idiotype-specific mAb (JS-10) also comodulates CD19.

Modulation using anti-Ig mAb reduces binding of 15 CD19-specific mAbs. 10 mAbs were obtained from the Fourth International Leukocyte Workshop, Vienna (B-C3, CLB-CD19, B43, B-G4, B-Ly3, HD37, HD237, B4, AB1, and BU-12). Five mAbs were prepared in our laboratory (J3-129, J3-144, and J4-166), including two (J4-35 and J3-119) from the workshop. Modulation of CD19 by J3-119 reduces binding of

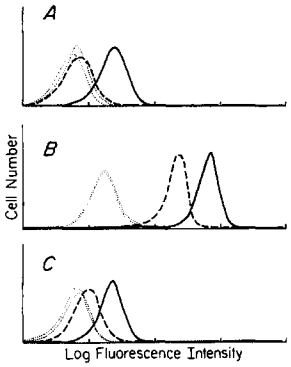

FIGURE 1. Incubation of B cells with anti-Ig mAb comodulates CD19. B cell lines Ramos (μ, λ), SKLY18 (γ, λ), and BALM-3 (γ, κ) are studied in *A*, *B*, and *C*, respectively. Cells are incubated with the appropriate purified anti-Ig mAb (10 μg/ml) or with an isotype-matched negative control mAb (anti-CD4) at 37°C. At 20 h, indirect immunofluorescence assay indicates loss of >95% of sIg from anti-Ig-treated cells. Directly fluoresceinated mAbs measure CD19 on control and anti-Ig-treated samples (*solid and dashed thick lines*, respectively). Binding of fluoresceinated nonreactive anti-CD4 mAb to both samples is indicated by overlapping sets of dotted lines.

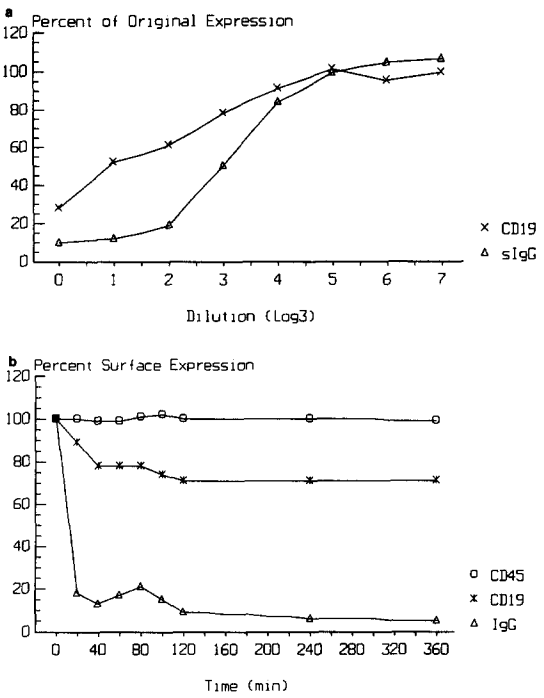

FIGURE 2. (*a*) Comodulation of CD19 by anti-Ig mAbs is concentration dependent. SKLY18 cells are incubated for 20 h with increasing dilutions ($\log_3^{-x}$) of either λ chain-specific (J6-17) or CD4-specific mAbs (J5-20), beginning at a purified mAb concentration of 10 μg/ml. Directly fluoresceinated mAbs measure expression of sIgG γ chain (HB43) and CD19 (J3-119). The ratio of binding of these mAbs to anti-Ig- and anti-CD4-treated cells (mean channel of fluorescence) at each dilution is plotted. Maximum efficiency of sIg modulation as measured using anti-γ mAb is only 90% in this experiment. (*b*) Anti-Ig mAbs induce concurrent changes in expression of sIg and CD19. SKLY18 cells are incubated with γ chain-specific mAb HB43 at 37°C. Expression of sIgG λ chain (J6-17), CD19 (J3-119), and CD45 (J-33) is measured at indicated times using directly fluoresceinated mAbs and monitored on the FACS. Identical results for modulation of sIgG are obtained using the modulating mAb (HB43) in an indirect immunofluorescence assay. Anti-Ig modulation has no effect on expression of CD45.

the other 14 CD19-specific mAbs. These 15 mAbs detect at least three CD19 epitopes, as determined by binding to lymphocytes of baboon and macaque and competitive inhibition studies with B4-FITC.

Modulation of sIg by anti-Ig mAb comodulates CD19 on nine of nine mature B cell lines and on normal tonsil B cells. Incubation with mAbs specific for both CD19 and sIg enhances the rate and extent of CD19 modulation. When 18 surface antigens are studied on nine anti-Ig-treated mature B cell lines using directly fluoresceinated mAbs (HLA I, HLA II, CD9, CD10, CD19, CD20, CD22, CD45, CD45R, CD46, CD48, CD71, p23, p35,90, p60, p124, p129, and p160,200), only expression of CD19 is consistently and significantly reduced.

Comodulation of CD19 and sIg is unidirectional. Modulation of CD19 by anti-CD19 mAb on nine of nine cell lines has no effect on expression of sIg or other antigens.

Higher concentrations of anti-Ig mAb increase the comodulation of CD19 (Fig. 2 *a*). sIgG (HB43) and CD19 (J3-119) are measured on SKLY18 cells incubated with increasing dilutions ($\log_3^{-x}$) of λ chain-specific mAb (J6-17) or isotype-matched CD4-specific mAb (J5-20). Changes in expression of sIg and CD19 also occur concurrently with time during anti-Ig modulation, suggesting that these are parallel rather than serial events. In Fig. 2 *b*, 87 and 95% of sIgG, and 22 and 29% of CD19 are lost from SKLY18 cells during anti-Ig modulation at 40 and 360 min, respectively. Expression of CD45 is not affected.

Anti-Ig mAb (HB43) induces capping of fluoresceinated mAb specific for CD19 but not other antigens (CD20 [B1], CD45 [J-33], and CD46 [J-48]). Anti-CD19 mAbs alone do not cap under these conditions. Two-color studies using fluoresceinated

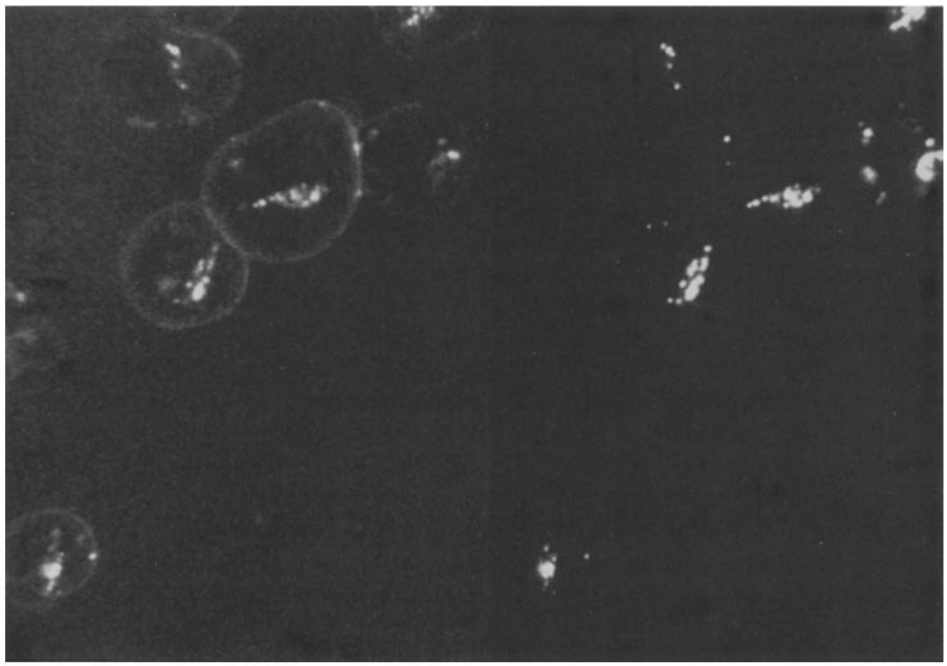

FIGURE 3. Anti-Ig and anti-CD19 antibodies cocap. SKLY18 cells are incubated with Texas red-conjugated goat anti-human λ chain-specific antibody and with fluoresceinated anti-CD19 mAb (J3-119) for 70 min at 37°C. The same field is analyzed for fluorescein (*left*) and Texas red (*right*) stain. Field depth is 1 μm. The two sets of caps overlap, and most appear to be within cells. Some anti-CD19 mAb remains on the cell surface.

anti-CD19 mAb (J3-129, J3-144, or J3-119) and Texas red-conjugated anti-Ig antibody show that anti-Ig and anti-CD19 cocap (Fig. 3), but anti-CD45 (J-33), anti-Ia (J-70), anti-p35,90 (JS-56), and anti-p23 (JS-64) do not. Not all of each anti-CD19 mAb caps, consistent with the comodulation data. Labeled anti-CD19 and anti-Ig antibodies appear within capped cells, suggesting that they are internalized.

Discussion

Anti-Ig mAbs cause rapid, specific, concentration-dependent, and reversible unidirectional comodulation of the CD19 phosphoglycoprotein on all mature B cells studied. Anti-Ig mAbs cause specific cocapping and apparent internalization of sIg and CD19, indicating both physical and functional interactions between these two B cell-specific and pan-B surface molecules, or between CD19 and some other molecule associated with sIg. Previously (16), anti-CD19 mAbs were shown to inhibit the rapid calcium ion changes and cellular proliferation caused by antibodies to sIg.

Comodulation of CD19 by anti-Ig mAbs is not due to serologic crossreactivity between CD19 and sIg; mAbs specific for the γ, μ, κ, and λ chains of sIg and at least one idiotype-specific mAb all produce this effect. Comodulation of CD19 is observed on all mature B cells studied, indicating that it is not a unique feature of one or two cell lines. Binding of 15 of 15 CD19-specific mAbs that identify at least

three different determinants on CD19 is reduced under these conditions, indicating that the effect is not due to an idiosyncratic property of a single anti-CD19 mAb. Of 18 antigens studied, CD19 is the only one comodulated by anti-Ig mAb. The parallel loss of sIg and CD19 caused by anti-Ig mAb with time and with varying antibody concentration, and the cocapping data, suggest that these are simultaneous rather than serial events.

The observation that sIg and CD19 comodulate with anti-Ig but not anti-CD19 mAb was unexpected. One explanation is that sIg and CD19 associate only in the presence of antigen or antigen substitutes. Such ligands would induce a change in sIg, possibly conformational, enabling it to bind CD19. Independent expression of sIg and CD19 is also indicated by the presence of CD19 on sIg^- pre-B cells, large excess of sIg over CD19 on many B cells, and ability of 10 μg/ml of anti-Ig mAb to produce virtually complete modulation of sIg but not CD19.

These studies demonstrate both functional and physical interactions between sIg and CD19. The long cytoplasmic tail of CD19 (148 amino acids) (14) relative to sIg (four amino acids for sIgM) (1, 2) suggests that CD19 could facilitate signal transduction when antigen is bound to sIg.

Summary

The pan-B and B cell–specific sIg and CD19 antigens are functionally and physically associated in the presence of anti-Ig mAb. Incubation of B cells with anti-Ig antibodies causes rapid, specific, reversible, concentration-dependent, and unidirectional comodulation of CD19 on every mature B cell studied. Comodulation is produced by mAbs specific for the γ, μ, κ, and λ chains of Ig, and by at least one idiotype-specific mAb. Comodulation is observed using 15 CD19-specific mAbs that detect at least three different CD19 epitopes. Of 18 surface antigens studied, only CD19 is comodulated. Loss of sIg and CD19 occurs concurrently during anti-Ig modulation and demonstrates a comparable dependence on anti-Ig concentration, suggesting that these are parallel rather than serial events. Incubation with anti-Ig specifically cocaps and suggests internalization of anti-CD19 mAb. Comodulation of sIg and CD19 by anti-Ig but not anti-CD19 mAbs suggests that ligand binding enables sIg to then interact with CD19. We propose that CD19 is a component of the B cell antigen receptor and suggest that it could facilitate signal transduction by sIg-antigen complexes.

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