Role of Virion-associated Glycosylphosphatidylinositol-linked Proteins CD55 and CD59 in Complement Resistance of Cell Line–derived and Primary Isolates of HIV-1

By Mohammed Saifuddin,* Charles J. Parker,‡ Mark E. Peeples,* Miroslaw K. Gorny,§ Susan Zolla-Pazner,§ Mahmood Ghassemi,* Isabelle A. Rooney,‖ John P. Atkinson,¶ and Gregory T. Spear*

*From the *Department of Immunology/Microbiology, Rush University, Chicago, Illinois 60612; ‡Veterans Affairs Medical Center, Salt Lake City, Utah 84148; §Department of Pathology, New York University Medical Center, New York 10016; ‖Department of Medical Biochemistry, University of Wales College of Medicine, Cardiff CF4 4XN, Wales; and ¶Department of Medicine, Washington University School of Medicine, St. Louis, Missouri 63110*

Summary

This study investigates whether cell-derived glycosylphosphatidylinositol–linked complement control proteins CD55 and CD59 can be incorporated into HIV-1 virions and contribute to complement resistance. Virus was prepared by transfection of cell lines with pNL4-3, and primary isolates of HIV-1 were derived from patients' PBMCs. Virus was tested for sensitivity to complement-mediated virolysis in the presence of anti-gp160 antibody. Viral preparations from JY33 cells, which lack CD55 and CD59, were highly sensitive to complement. HIV-1 preparations from H9 and U937 cells, which express low levels of CD55 and CD59, had intermediate to high sensitivity while other cell line–derived viruses and primary isolates of HIV-1 were resistant to complement-mediated virolysis. Although the primary isolates were not lysed, they activated complement as measured by binding to a complement receptor positive cell line. While the primary isolates were resistant to lysis in the presence of HIV-specific antibody, antibody to CD59 induced lysis. Likewise, antibody to CD55 and CD59 induced lysis of cell line–derived virus. Western blot analysis of purified virus showed bands corresponding to CD55 and CD59. Phosphatidylinositol-specific phospholipase C treatment of either cell line–derived or primary isolates of HIV-1 increased sensitivity to complement while incubation of sensitive virus with purified CD55 and CD59 increased resistance to complement. These results show that CD55 and CD59 are incorporated into HIV-1 particles and function to protect virions from complement-mediated destruction, and they are the first report of host cell proteins functioning in protection of HIV-1 from immune effector mechanisms.

Several studies have shown that host cell proteins can be incorporated into the membranes of budding HIV-1 virions. The incorporated proteins include class I and II MHC and CD11a/CD18 (1–4). Some of these virion-associated molecules may play a necessary functional role during cellular infection or in resistance to immune effector mechanisms. For example, host-derived receptors such as CD11a/CD18 on virions could facilitate adhesion of virus to target cells. Also, antibodies to MHC class I molecules can neutralize HIV (1), providing evidence for a critical role of these molecules in virus function. Studies have shown, however, that virions lacking class I (5) or CD11a/CD18 (6) are still infectious. Thus, it has not yet been clearly demonstrated whether incorporation of host cell proteins into virions can play a functional role in HIV-1 infection.

High levels of complement and anti-HIV antibody are present in the blood of infected persons, and therefore one might predict that virions can be lysed by the membrane attack complex (MAC)[1] since in previous in vitro studies, virus derived from the H9 cell line can be destroyed in this way (7, 8). However, during infection with HIV-1, up to 10^4–10^6 free virions per milliliter can be found in the plasma

[1] *Abbreviations used in this paper:* β_2m, β_2-microglobulin; CR, complement receptor; GPI, glycosylphosphatidylinositol; HI, heat inactivated; MAC, membrane attack protein; PI-PLC, phosphatidylinositol-specific phospholipase C.

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of infected persons (9, 10), suggesting that in vivo HIV virions may be resistant to complement-mediated virolysis. The goals of this study were to determine whether complement control proteins were incorporated by virions either from HIV-1–infected cell lines or from HIV-1–infected primary patient isolates, and whether the proteins functioned in resistance of virions to complement activation leading to lysis.

The results of this study indicate that glycosylphosphatidylinositol (GPI)-linked proteins CD55 and CD59 are present on HIV-1 and provide resistance to complement-mediated lysis. This is the first demonstration that virion-associated, host cell–derived proteins function in the resistance of cell-free virus to immune effector mechanisms.

Materials and Methods

Cells. The T-lymphocytic H9 (HTB 176), the promonocytic U937 (CRL 1593), and the B-lymphoblastic CESS (TIB 190) cell lines were obtained from the American Type Culture Collection (Rockville, MD). The B-lymphoblastic AA-2 cell line (11) was obtained from the National Institutes of Health (NIH) AIDS Research and Reference Reagent Program (contributed by Michael Hershfield, NIH, Rockville, MD). The B-lymphoblastic JY cell line and the mutant JY33 subline (deficient in GPI-linked proteins expression) were obtained from Dr. Timothy Springer (Dana Farber Cancer Institute, Boston, MA). The T-lymphoblastic HPB-ALL cell line (12) was also used in this study. Cells were grown in RPMI-1640 (Whittaker Bioproducts, Walkersville, MD) supplemented with 10% heat-inactivated FCS (Hyclone Laboratories, Logan, UT).

All cell lines were tested and found to be negative for mycoplasma contamination using the GEN-PROBE® Mycoplasma T. C. Rapid Detection System (Gen-Probe Inc., San Diego, CA).

Complement, Antibodies, and Proteins. Sera from HIV antibody–negative AB^+ donors were aliquoted and frozen at −70°C for use as a source of complement. In some experiments, serum was heat inactivated (HI) at 56°C for 60 min to destroy complement activity, and it was used as negative complement control.

A goat antiserum to a baculovirus-derived $HTLV_{IIIB}$ (BH10) gp160 was obtained from the NIH AIDS Research and Reference Reagent Program (contributed by Robert Gallo, National Cancer Institute, Rockville, MD) and used as a source of anti-HIV antibody. Polyclonal rabbit antisera to CD55 and CD59 were prepared as previously described (13, 14). All antisera used as sources of antibody were HI at 56°C for 45 min. The anti-CD55 and anti-CD59 antisera were absorbed with JY33 mutant cells to remove reactivity against other cellular proteins. Mouse mAb to human CD55 (clone No. BRIC110) and CD59 (clone No. MEM43) antigens were obtained from Tago (Burlingame, CA) and Biodesign (Kennebunk, ME), respectively, and horseradish peroxidase–conjugated goat anti–mouse Ig was obtained from Amersham Corp. (Arlington Heights, IL). Human CD55 and CD59 proteins were prepared from erythrocytes as described previously (15). Anti-CR2 mouse mAb (clone No. OKB7, IgG2b) was obtained from Ortho Diagnostic Systems, Inc. (Raritan, NJ).

Viruses. A full-length, infection-competent HIV-1 clone pNL4-3 (16) obtained from the NIH AIDS Research and Reagent Program (contributed by Malcolm Martin, NIH, Rockville, MD) was used to transfect seven human cell lines by a DEAE-dextran method (17). At 4 d posttransfection, cell supernatant was filtered (0.45 μm), aliquoted, and stored at −70°C as cell-free virus stock.

Five primary isolates of HIV-1 (JC50, MG47, MP49, PE51, and CJ48) were derived from asymptomatic HIV-1–infected subjects. Primary isolates were generated by coculture of PBMCs from infected and uninfected donors according to the method described by Gartner and Popovic (18). PBMCs from infected and uninfected subjects were stimulated separately for 3 d with phytohemagglutinin (5 μg/ml) and then cocultured at a 1:3 ratio in the presence of IL-2. Supernatants were harvested every 2nd d after the 7th d of culture, and aliquots stored at −70°C as virus stocks. The level of p24 antigen in virus preparations was determined by ELISA (Coulter Co., Hialeah, FL).

Complement-mediated Virolysis. To determine the sensitivity of HIV to complement, 20-μl aliquots of virus containing 10 ng p24/ml were treated with complement or HI serum (final dilution 1:10 unless otherwise specified) either alone or with anti-gp160 antiserum for 60 min at 37°C. Virolysis resulting from antibody-mediated complement activation was calculated by measuring the release of HIV-1 p24 antigen by ELISA (Coulter Co.) (7). A minimal background virolysis (0–4%) caused by complement alone was subtracted from each value obtained from complement plus antibody-mediated lysis. Percent virolysis was calculated by the following formula: [experimental lysis (no detergent) − untreated virus (background)] / [experimental lysis (with detergent) − untreated virus] × 100.

To demonstrate the presence of CD55 and/or CD59 on HIV-1, virus preparations were treated with complement (final dilution 1:10 unless otherwise specified) plus different dilutions of anti-CD55 or anti-CD59 antiserum.

Cell-binding Assay for Detecting Activation of Complement by HIV. To determine the ability of HIV-1 primary isolates to activate complement and thereby bind to complement receptor (CR)-positive HPB-ALL cells, aliquots of 30 μl of each isolate (containing 10 ng p24/ml) were incubated with complement or HI serum (final dilution 1:10) or complement plus anti-gp160 antiserum (final dilution 1:50) for 60 min at 37°C. Virus was then added to a pellet of 1.5×10^6 HPB-ALL cells, mixed and incubated for 60 min on ice. Cells were washed three times to remove unbound virus, resuspended in 500 μl of 0.5% Triton X-100 and cell lysates tested for the levels of cell-bound HIV-1 p24 antigen by ELISA. In other experiments designed to determine the mechanism of complement-mediated binding of HIV-1, HPB-ALL cells were pretreated with anti-CR2 antibody (OKB7) or isotype IgG control before incubation with virus.

Detection of CD55 and/or CD59 on HIV by Western Blotting. Cell-free virus preparations were derived from pNL4-3 transfected HPB-ALL cells. Virus was concentrated approximately 100-fold by ultracentrifugation at 140,000 *g* through 20% glycerol for 3 h and then purified by sucrose gradient (25–55% wt/wt) ultracentrifugation (275,000 *g*) for 18 h as described previously (19, 20). The fractions which had an approximate density of 1.16 g/ml and contained the peak level of p24 were resuspended in lysis buffer (0.05 M Tris-HCl pH 7.2, 0.15 M NaCl, 0.1% SDS, 1.0% deoxycholate, 1 mM phenyl methylsulfonyl fluoride) and subjected to 12% polyacrylamide-SDS (0.1%) gel electrophoresis (21) under nonreducing conditions. A mock virus control was prepared from uninfected HPB-ALL cell supernatants after the identical procedures of virus concentration and sucrose gradient purification. Purified CD55 and CD59 proteins were used as positive controls. The total amount of protein in the purified virus preparation was estimated by using the BCA protein assay reagent (Pierce Chemical Co., Rockford, IL). Proteins were electrotransferred to nitrocellulose membranes overnight at 4°C. Membranes were incubated with mouse anti-CD55 or anti-CD59 mAb (final concentration = 0.75 μg/ml) washed and further incubated with goat anti–mouse Ig-conjugated

horseradish peroxidase (dilution 1:2,500). Antigen-reactive bands were detected using enhanced chemiluminescence detection reagents (Amersham Corp., Arlington Heights, IL) followed by exposure to x-ray film (Fuji Photo Film Co., Japan).

Treatment of HIV with Phosphatidylinositol-specific Phospholipase C (PI-PLC). To remove GPI-linked proteins, HPB-ALL cell-derived virus or the primary isolates MG47 or MP49 (20 μl containing 10 ng p24/ml) was incubated with PI-PLC (gift from Dr. M. Low, Columbia University, New York) (final concentration = 1–6 U/ml) for 60 min at 37°C, and then PI-PLC was removed by ultracentrifugation through 20% glycerol. Virus was then incubated with complement plus anti-gp160 antiserum for 60 min at 37°C, and virolysis was determined by p24 ELISA.

Reconstitution of Virus with CD55 and CD59 Protein. To determine the effect of reconstitution of virions with exogenous complement control protein(s), viral preparations (20 μl containing 10 ng p24/ml) derived from U937 and JY33 cell lines or PI-PLC treated viruses were incubated with or without CD55 or CD59 (final concentration = 3 μg/ml) for 60–90 min at 37°C. Virus was then tested for sensitivity to virolysis in the presence of complement plus anti-gp160 antiserum.

Results

Sensitivity of Cell Line–derived HIV to Complement. Previous studies had shown that HIV-1 derived from H9 cells was sensitive to complement-mediated virolysis in the presence of antienvelope antibody (22). To determine whether host cell components might affect the sensitivity of virus to complement in the presence of anti-gp160 antibody, seven cell lines, each expressing different levels of complement control proteins (7), were transfected with the infectious plasmid pNL4-3. This method of preparing virus was used to avoid the possibility that some substrains of virus might grow preferentially in different cell lines. Virions derived from the cells were then tested for sensitivity to complement-mediated virolysis.

Viral preparations from the different transfected lines exhibited a broad range (2–99% virolysis) of sensitivity to complement in the presence of a 1:50 dilution of anti-gp160 antibody. As previously reported (22), moderate levels (34%) of complement-mediated lysis of H9-derived HIV-1 occurred in the presence of antiviral antibody (Fig. 1). Minimal lysis (3%) was observed with virus derived from JY cells that express high levels of CD55 and CD59, while substantially higher levels of lysis (72%) were observed with virus derived from mutant JY33 cells that are deficient in expression of GPI-linked proteins including CD55 and CD59 (Fig. 1). These results are consistent with the possibility that host cell GPI-linked proteins were incorporated into JY-derived virions and inhibited complement-mediated virolysis. This hypothesis is supported by the observation that H9-derived virus is intermediately sensitive to complement (Fig. 1) since our previous studies have shown that the H9 cell line has moderate to low levels of expression of CD55 and CD59 (7). Little or no virolysis (≤1%) was observed with all cell line–derived virus in the presence of complement alone, except JY33-derived virus, where up to 4% lysis occurred. Additionally, antibody plus HI serum caused no lysis of virus (≤1%) (not shown).

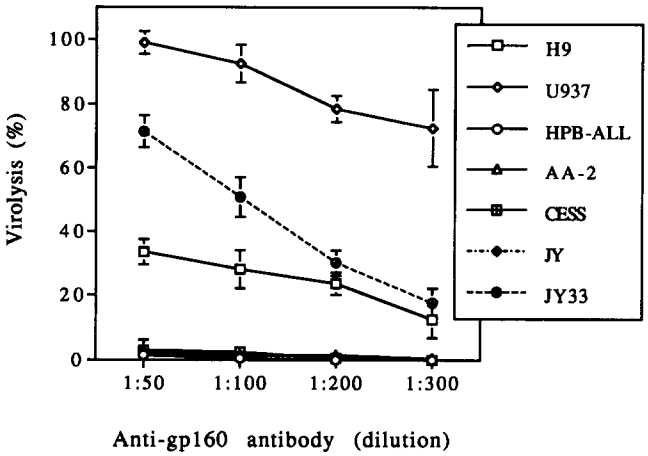

Figure 1. Sensitivity of cell line–derived HIV-1 to complement-mediated virolysis. Viral preparations (20 μl containing 10 ng p24/ml) derived from pNL4-3–transfected cell lines, were incubated with complement plus goat antiserum to gp160. Viral preparations were also treated with growth medium and Triton X-100 to determine 0 and 100% lysis, respectively. Percentage of virolysis was calculated by measuring the release of p24 caused by complement activation compared to total p24 content released by detergent. Each value represents the mean ±SD of three experiments.

Viruses derived from HPB-ALL, AA-2, and CESS cells were resistant to complement (2–3% lysis), even at the highest concentration of anti-gp160 antibody (Fig. 1). Since these cell lines express high levels of CD55 and CD59 (7), the complement resistance of virus derived from these cells could result from incorporation of CD55 and CD59 into the virions. Interestingly, virus derived from the U937 cell line was highly sensitive to complement-mediated lysis, even in the presence of the highest dilution (1:300) of anti-gp160 antibody (Fig. 1). The U937 cells express low levels of CD55 and CD59 (7) and may contain a dysfunctional form of CD59 (23).

Activation of Complement by Primary Isolates of HIV. Since previous studies have shown that cell line-derived virus and primary isolates of HIV may have different properties, five primary isolates of HIV-1, derived from five separate HIV-infected patients, were tested for their sensitivity to complement in the presence of anti-gp160 antibody. All five isolates were resistant (3–7% lysis) to complement-mediated virolysis in the presence of high levels of anti-HIV antibody while virus derived from JY33 cells was sensitive (Fig. 2). Since the primary isolates of HIV-1 were found resistant to complement-mediated lysis, it was possible that the anti-viral antibody used in these experiments did not bind to the intact virions. To test this, the primary isolates were assessed for complement activation using a cell-binding assay. Previous studies have shown that complement-treated virus will bind to $CR2^+$ cells under conditions which do not favor virolysis (24). Cells were incubated with virus that was pretreated with complement, HI serum or complement plus anti-gp160 antibody, and binding to $CR2^+$ HPB-ALL cells was assessed. All five isolates activated complement in the absence of antibody as has previously been observed with cell line–derived virus (24), but activation was greater in the presence

of anti-gp160 antibody (Fig. 3). In other experiments designed to determine the mechanism of complement-mediated binding of HIV-1 to HPB-ALL cells, it was observed that binding of three primary isolates (MG47, MP49, and CJ48) was inhibited by 73–86% when HPB-ALL cells were preincubated with 10 μg/ml of OKB7 antibody (data not shown), indicating that complement-mediated viral binding to HPB-ALL cells occurred via CR2. There was no inhibition of binding of HI serum–treated virus. Thus, while primary isolates activated complement in the presence of anti-gp160 antibody, these viruses were resistant to lysis (Fig. 2).

Association Of CD55 and CD59 with Cell Line–derived and Primary Isolates of HIV-1. To directly demonstrate whether CD55 and/or CD59 were associated with virions, viral preparations were treated with complement plus antibody to CD55 or CD59, and virolysis was assessed. The antisera to CD55 and CD59 that were used in these experiments were both highly specific for the appropriate proteins, as determined by Western blot. High levels of lysis (71%) of the JY cell–derived virus were observed after treatment with complement plus 1:60 dilution of anti-CD59 antibody (Fig. 4), indicating that CD59 protein was associated with virions. Anti-CD59 antibody caused minimal lysis of the complement sensitive virus derived from the JY33 cell line, which is deficient in CD59 (Fig. 4). In contrast to anti-CD59 induced lysis, low levels of lysis (2%) of JY-derived virus was obtained with 1:50 dilution of anti-CD55 antibody (not shown).

Experiments with viruses from other cell lines such as HPB-ALL, AA-2, CESS, and H9 gave similarly higher levels of lysis (40–83%) with 1:50 dilution of anti-CD59 antibody (Table 1). A relatively low level of lysis (10%) of U937-derived virus was observed with anti-CD59 antibody. In contrast to anti-CD59–induced virolysis, only 1–2% lysis of HPB-ALL–, AA-2–, and CESS-derived viruses was observed with 1:50 dilution of anti-CD55 antibody while H9 and U937-derived viruses were lysed (Table 1). These results clearly show that CD59 is associated with virions since it can act as a target for virolysis. Although lysis was lower, anti-CD55 also clearly

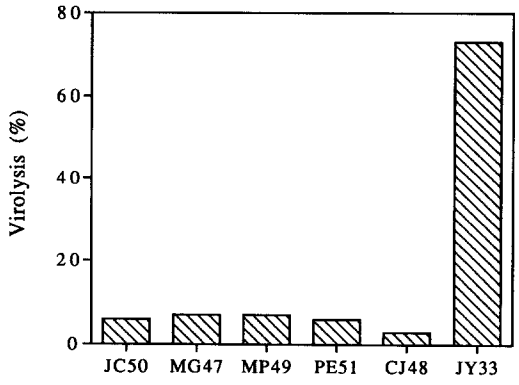

Figure 2. Sensitivity of primary isolates of HIV-1 to complement-mediated virolysis. Five primary isolates (20 μl containing 10 ng p24/ml) were incubated with human complement plus anti-gp160 antibody (final dilution 1:50), and the percentage of virolysis was calculated. pNL4-3–transfected JY33-derived virus was used as a positive lysis control. Results are representative of three separate experiments.

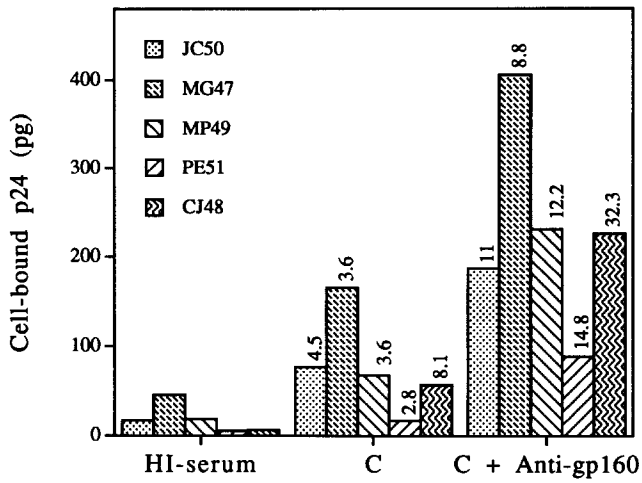

Figure 3. Complement-mediated binding of primary isolates of HIV-1 to CR2+ cells. Aliquots (30 μl containing 10 ng p24/ml) of five primary isolates were incubated with complement (*C*), heat-inactivated (*HI*) serum, or C plus anti-gp160 antibody (final dilution =1:50). The virus mixture was then incubated with 1.5 × 10^6 HPB-ALL cells for 60 min on ice. After washing, cells were lysed with detergent and tested for cell-bound HIV-1 p24 by ELISA. Numbers above the columns indicate fold increases of cell-bound p24 relative to HI serum-treated virus. Results are representative of three similar experiments.

lysed virus from H9 and U937 cells and thus CD55 can be associated with virions. The lack of lysis of JY-, HPB-ALL–, AA-2–, and CESS-derived virus preparations observed with anti-CD55 antibody could result either from lack of CD55 or from a protective effect from other viral factor(s), including CD59.

Complement-mediated virolysis (14–25%) of all five primary isolates was achieved after treatment with anti-CD59 antibody while low, but significant ($P < 0.05$, ANOVA) virolysis (2–5%) of three out of five primary isolates was observed

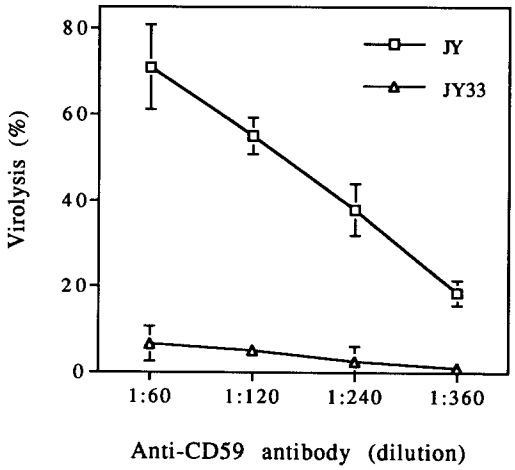

Figure 4. Virolysis of HIV-1 initiated by antibody to CD59. Virus derived from the pNL4-3–transfected JY and JY33 cell lines were incubated with complement plus serial dilutions of rabbit anti-CD59 antiserum for 60 min at 37°C, and the percentage of virolysis was determined. Each value represents the mean ±SD of three experiments.

Table 1. *Complement-mediated Lysis of Cell Line-derived HIV-1 Induced by Anti-CD55 and Anti-CD59 Antibody**

	Percentage of virolysis				
	H9	U937	HPB-ALL	AA-2	CESS
Anti-CD55	12 ± 3.0	20 ± 14.0	1 ± 1.0	2 ± 1.0	1 ± 1.0
Anti-CD59	40 ± 7.0	10 ± 4.0	83 ± 3.0	39 ± 10.0	55 ± 8.0
C alone	1 ± 0.9	1 ± 0.6	0 ± 0.0	1 ± 1.0	0 ± 0.0

* HIV-1 clone pNL4-3–transfected cell line–derived viruses were incubated with complement (final dilution 1:5) either alone or with anti-CD55 or anti-CD59 antibody (final concentration = 1:50). Release of core protein, p24 was measured by ELISA. Each value represents the mean ±SD of three experiments.

with anti-CD55 (Fig. 5). Virolysis induced by anti-CD55 and anti-CD59 antibodies directly demonstrated that both GPI-linked proteins were associated with the primary isolate virions and suggested that their presence could be related to the resistance of the virus to complement.

HIV-1 associated CD55 and CD59 were also assessed on Western blots of purified virus. Both proteins were detected on sucrose density gradient–purified virions (Fig. 6), which were derived from pNL4-3–transfected HPB-ALL cells. These cells express relatively high levels of both CD55 and CD59 (7), and as shown above, virus from these cells was susceptible to lysis with anti-CD59 antibody but resistant to lysis with anti-CD55. In contrast, no CD55 was detected in a mock virus preparation from uninfected HPB-ALL cells, while a faintly detectable band was observed when blots were probed with anti-CD59. These results indicate that both CD55 and CD59 are present on virions.

Effects of Exogenous CD55 and CD59 on HIV Complement Sensitivity. Previous studies have shown that exogenously supplied GPI-linked proteins can become incorporated into lipid membranes and function in complement resistance of erythrocytes (13, 25). Since the above experiments suggested that complement resistance of HIV-1 may be at least in part caused by the presence of viral associated CD55 and CD59, complement-sensitive viruses from cells that are completely (JY33) or partially (U937) deficient in CD55 and CD59 (7) were incubated with exogenous CD59 and analyzed for their susceptibility to complement-mediated lysis. The susceptibility of JY33- and U937-derived viruses were substantially reduced after incubation with CD59 (Fig. 7 *A*). Similarly, susceptibility of U937-derived virus was also reduced after incubation with CD55 and CD59 (Fig. 7 *B*). Thus, comple-

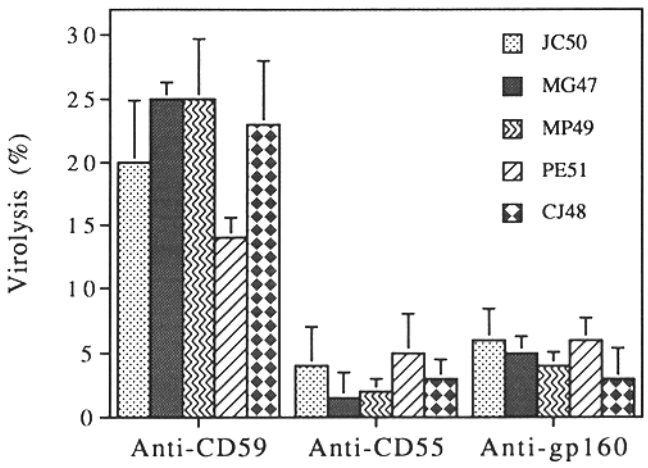

Figure 5. Virolysis of HIV-1 primary isolates initiated by antibodies to CD55 and CD59. Aliquots of each isolate were incubated with complement (final dilution 1:5) plus anti-CD59 or anti-CD55 antiserum (final dilution =1:50). Each isolate was also incubated with complement plus anti-gp160 antibody (final dilution =1:50). Percentage of virolysis was determined as described in the legend to Fig. 1. Each value represents the mean ±SD of three experiments.

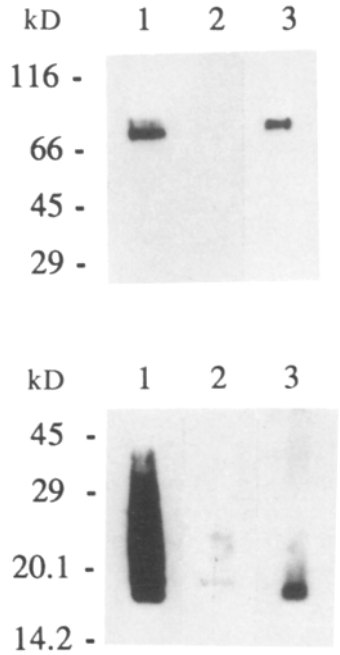

Figure 6. Detection of CD55 and CD59 on HIV-1 by Western blotting. Cell-free HIV-1 derived from pNL4-3–transfected HPB-ALL cells and a mock virus preparation derived from uninfected HPB-ALL were purified by sucrose density gradient ultracentrifugation. Proteins were separated on a 12% SDS-polyacrylamide gel, electrotransferred onto a nitrocellulose membrane, incubated with mouse monoclonal primary antibody, washed, and incubated with goat anti–mouse Ig-conjugated horseradish peroxidase. Protein bands were visualized by treating the blots with enhanced chemiluminescence detection reagents before exposure to x-ray film. The upper panel is stained with monoclonal anti-CD55 antibody: Lanes *1* and *3* contain 10 μg of protein of purified virus and 10 ng of purified CD55, respectively. Lane *2* contains a volume of purified mock virus that corresponds to the volume of virus used in lane *1*. The lower panel is stained with monoclonal anti-CD59 antibody: lanes *1* and *3* contain 1.2 μg of proteins of purified virus and 5 ng of purified CD59, respectively. Lane *2* contains a volume of purified mock virus that corresponds to the volume of virus used in lane *1*.

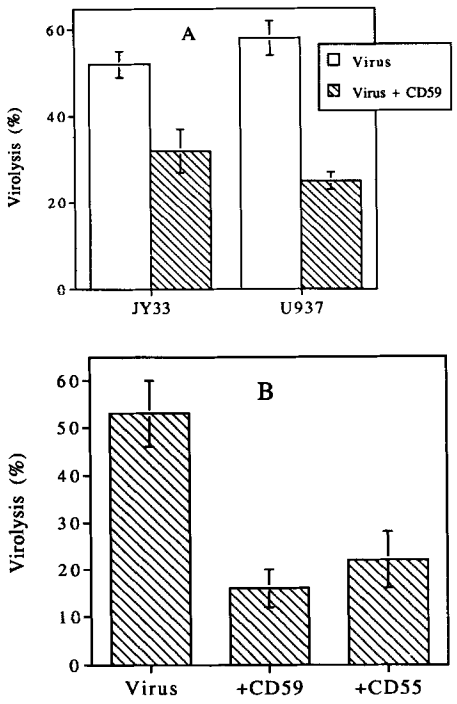

Figure 7. Inhibition of complement-mediated lysis of HIV-1 after incubation with purified CD55 and CD59 proteins. (*A*) Virus derived from pNL4-3 transfected JY33 and U937 cell lines were treated with or without CD59 (final concentration = 3 μg/ml) for 60 min at 37°C. JY33- and U937-derived viruses were then further incubated with complement (final dilution =1:40 and 1:60, respectively) plus anti-gp160 antibody (final dilution = 1:100) for 60 min at 37°C. The result shown is representative of three similar experiments and each value represents the mean ±SD of duplicate values in the representative experiment. (*B*) U937-derived virus was treated with or without CD55 or CD59 and susceptibility to gp160 antibody-initiated complement lysis was assessed. Each value represents the mean ±SD of two experiments.

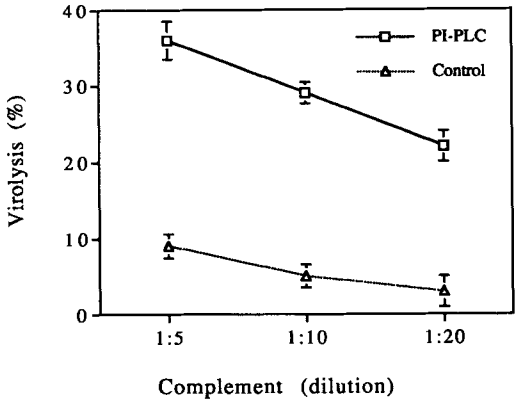

Figure 8. Increased complement sensitivity of HIV-1 primary isolates after treatment with PI-PLC. Aliquots of primary isolate MG47 were treated with buffer or buffer containing PI-PLC (final concentration = 6 U/ml) for 60 min at 37°C. Virus was then further incubated with serial dilutions of complement plus anti-gp160 antibody (final dilution =1:50) for 60 min at 37°C. Each value represents the mean ±SD of three experiments.

ment resistance of virus was increased by incorporation of exogenous CD55 or CD59.

Effects of PI-PLC Treatment and CD55/CD59 Reconstitution on HIV Complement Resistance. Since CD55 and CD59 are covalently bound to cell membranes by a GPI linkage, the MG47 primary isolate was treated with PI-PLC and analyzed for susceptibility to complement lysis in the presence of anti-gp160 antibody. Virolysis of PI-PLC–treated MG47 virus was substantially increased at all dilutions of complement compared to untreated control (Fig. 8), indicating that GPI-linked proteins, including CD55 and CD59, are present on virions and function in resistance of virus to complement. Cell lines H9 and HPB-ALL were also treated with PI-PLC, and they were analyzed for the presence of CD55 and CD59. Treatment with PI-PLC removed ~ 80–90% of CD55 and CD59 (not shown).

The above experiments indicated that host cell complement control proteins CD55 and CD59 are present on cell line–derived and primary isolates of HIV-1, and that cleavage of those proteins by PI-PLC could render the virus more susceptible to complement. The HPB-ALL–derived and primary isolates of HIV-1 (MG47 and MP49) that are resistant to complement were treated with PI-PLC and then ultracentrifuged through 20% glycerol to remove the PI-PLC. This treatment increased the complement sensitivity of these viral preparations from 1–4% to 17–35% virolysis (Fig. 9). The PI-PLC–treated viruses were then incubated with exogenously purified CD55 and CD59. As observed with the sensitive cell line–derived viral preparations, sensitivity of all three PI-PLC–treated viruses to complement were markedly reduced by treatment with purified CD59 (Fig. 9). In contrast to CD59, CD55 did not decrease virolysis although incubation with both proteins was more effective in protecting virions from lysis than CD59 alone.

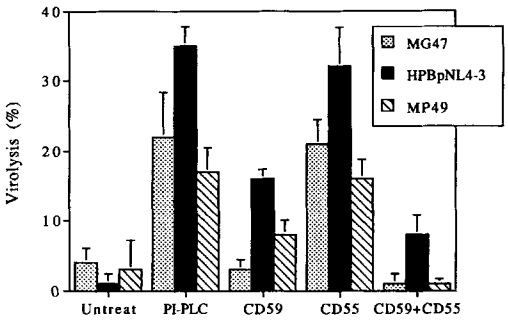

Figure 9. Treatment with PI-PLC increases complement sensitivity of primary HIV-1 isolates and a cell line-derived virus, and incubation with CD55 or CD59 restores complement resistance. pNL4-3–transfected HPB-ALL–derived virus and two primary isolates of HIV-1 (MG47 and MP49) were treated with PBS or PI-PLC (final concentration = 1.0 U/ml) for 60 min at 37°C and then ultracentrifuged through 20% glycerol to remove PI-PLC. Viruses were then further incubated with CD55 or CD59 or both (final concentration = 3 μg/ml) for 90 min at 37°C. Viral sensitivity to virolysis was analyzed after incubation with complement plus anti-gp160 antiserum. Each value represents the mean ±SD of two experiments.

Discussion

Complement control proteins constitute a mechanism that regulates a balance between the destruction of foreign organisms and the protection of the host against complement-mediated injury (26, 27). Complement pathway regulators such as decay accelerating factor (DAF, CD55) prevent formation of C3/C5 convertases and accelerate their decay (28), while cell surface membrane cofactor protein, (MCP, CD46) complement receptor type 1 (CD35), and plasma constituents factor H and C4 binding protein act as cofactors for Factor I–mediated cleavage of C3b and C4b (29, 30). The membrane inhibitor of reactive lysis (MIRL, CD59) inhibits formation of the cytolytic MAC (13, 31, 32). Of these inhibitors, CD55 and CD59 are anchored to the cell membrane by a GPI linkage. The current study was undertaken to investigate whether HIV-1 virions incorporate CD55 and CD59 into their membrane and thereby gain protection from MAC-mediated lysis.

Initial experiments performed in this study were consistent with the hypothesis that host cell–derived CD55 and CD59 were present on virions since there was an inverse relationship between expression of those proteins by host cells and complement sensitivity of virus from those cells. Thus, virus from JY33 cells that lacked CD55 and CD59 were highly sensitive to complement, virus from H9 cells that expressed low levels of CD55 and CD59 had intermediate sensitivity to complement, and virus from cells that expressed high levels of CD55 and CD59, including JY, HPB-ALL, AA-2, and CESS, were resistant to lysis. The exception to this pattern was virus derived from U937 cells, which expressed levels of CD55 and CD59 similar to H9 cells but was highly sensitive to lysis. The reason for the high susceptibility of U937-derived virus could result from one or more factors including (*a*) low expression of CD55 and CD59 by U937; (*b*) possible expression of nonfunctional CD59 by U937 (23); (*c*) low expression of other regulators of complement activation such as CD46 that could be incorporated into virions; or (*d*) expression of higher levels of gp160 on virions from U937. Flow cytometric analysis, however, indicated that CD46 expression was similar among H9, JY33, and U937 cells (not shown), suggesting that differences in CD46 levels on virus may not account for the differing complement sensitivity of virus derived from these cells. The mechanism responsible for sensitivity of U937 virus is currently being investigated.

The results obtained in this study clearly demonstrate the presence of both CD55 and CD59 on virions since immunoblots showed bands for both CD55 and CD59. Additionally, antibody to CD59 induced substantial lysis of virions from JY and other cell lines, as well as from primary isolates. A somewhat different lysis pattern was obtained with antibody to CD55. This antiserum induced significant lysis of virions from U937 and H9 cells expressing low levels of CD55 and CD59, and essentially no lysis of virions from JY, HPB-ALL, AA-2, and CESS cells expressing high levels of CD55 and CD59 and low lysis of primary isolates of HIV-1. The difference in degree of lysis caused by the anti-CD55 and anti-CD59 antisera could result from several conditions including (*a*) a higher relative efficacy of CD59 in viral resistance to lysis coupled with an ability of anti-CD59 antibody to serve as an activator of complement as well as an inhibitor of CD59 function; (*b*) general lower expression of CD55 than CD59 by virus; or (*c*) lower potency of the anti-CD55 antiserum.

As described above, it was shown that primary isolates were resistant to complement-mediated lysis, at least in part because of the presence of CD55 and CD59 on the virions, and yet-activated complement. It has been shown that after activation of complement, the breakdown products C3dg and iC3b form on intact virions. As has been previously reported, instead of viral inactivation, complement can enhance infection of HIV-1 by enhancing binding of virions to cells through interaction with complement receptors (24, 33–35). This can occur under the condition of high levels of complement in the absence of antibody or low levels of complement in the presence of antibody. Thus, GPI-linked proteins on virions may facilitate enhancement of HIV-1 infection by inducing resistance to virolysis.

Both CD55 and CD59 proteins are anchored by GPI linkage and treatment of complement-resistant virus with PI-PLC conferred a phenotypic change to complement sensitive virus. This supports the hypothesis that the complement resistance of HIV-1 resulted from the presence of host cell–derived, GPI-linked complement regulatory proteins on the virions. Although complement sensitivity of PI-PLC–treated viruses was increased by more than fivefold in the presence of anti-gp160 antibody, the level of sensitivity obtained was still low compared to sensitive virus derived from the mutant JY33 line, which lacks both CD55 and CD59. This difference could be caused by incomplete removal of GPI-anchored proteins from virions by PI-PLC since by using flow cytometry we determined that the PI-PLC removed most (80–90%) but not all GPI-linked proteins on cells. Susceptibility of GPI-linked proteins to PI-PLC has been shown to vary due to several factors including the type of cells or surfaces to which proteins are attached (36), interactions with other membrane components, or modification of the structure of the GPI anchor (37).

An important observation in this study was that treatment of complement-sensitive or PI-PLC–treated viruses with exogenous human CD55 and/or CD59 increased complement resistance. Similar results have been observed using other experimental models. For example, treatment of rat oligodendrocytes with human erythrocyte-derived CD59 (38) or guinea pig erythrocytes with seminal plasma-derived CD59 (39) protected cells from lysis by human complement. Similarly, reconstitution of erythrocytes from patients with paroxysmal nocturnal hemoglobinuria with purified CD55 (25) or CD59 (13) ameliorated their complement sensitivity.

Earlier studies have reported that animal retroviruses are destroyed by human complement in the absence of antiviral antibody (40–42), whereas human retroviruses HIV and human T cell leukemia virus are resistant to complement (19, 43). The results of the current study suggest that the resistance of HIV and human T cell leukemia virus may result in part from the incorporation of host complement regulatory proteins into the virions. It is possible that the sensitivity of animal retroviruses grown in nonhuman cells could

result in part from the low regulatory activity of the animal complement control proteins against human complement (32, 44).

This study indicated that CD55 and CD59 retained their complement regulatory effect while incorporated on the surface of HIV-1 virions. Several other cellular proteins, including MHC class I and class II, β_2-microglobulin (β_2m), as well as the adhesion molecules leukocyte function–associated antigen-1 and intercellular adhesion molecule-1 associated with HIV virions have also been suggested to have functional effects on viral infectivity, and antibody to some of these molecules can neutralize virions (1). However, a recent report (5) showed that incorporation of MHC class I molecules are not essential for the infectivity of HIV, and that neutralization of virus by antibody to this molecule may be caused by a steric blocking effect. Similarly, it has been suggested that the association of β_2m with cytomegalovirus allows the virus to evade neutralization by anti-viral immune response (45). Alternatively this effect may again be simply due to nonspecific blocking of the neutralizing determinant of the virions by β_2m. In this regard, the current study provides evidence that cellular proteins incorporated on the virions remain functionally active and directly contribute resistance to immune-mediated attack. Whether this function would be essential for viral replication in vivo is not known.

In conclusion, this report documents that host cell complement control proteins CD55 and CD59 are incorporated into HIV-1 virions and contribute to the viral resistance to complement, and, as such, this is the first report to show a functional role for host cell–derived proteins. This mechanism is likely to contribute to the escape of virions from the complement system in vivo.

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Address correspondence to Dr. Mohammed Saifuddin, Department of Immunology/Microbiology, Rush University, 1653 West Congress Parkway, Chicago, IL 60612.

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