Human immunodeficiency virus type 1 incorporates both glycosyl phosphatidylinositol-anchored CD55 and CD59 and integral membrane CD46 at levels that protect from complement-mediated destruction

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Human immunodeficiency virus type 1 (HIV-1) can be either resistant or sensitive to complement-mediated destruction depending on the host cells. Incorporation of different levels of host cell CD46, CD55 and CD59 may account for this differential sensitivity to complement. However, it has not been determined whether CD46, CD55 and CD59 can all be incorporated at levels which protect virions. To determine whether each of these proteins can protect HIV-1, virions were derived from CHO cells expressing either human CD46, CD55 or CD59. Virions were shown to incorporate both glycosyl phosphatidylinositol (GPI)-anchored CD55 and CD59 as well as transmembrane CD46. Importantly, all three virus preparations were significantly more resistant to complement lysis than control virus. This study demonstrates that HIV-1 incorporates both transmembrane and GPI-anchored complement control proteins from host cells and that both types of protein increase complement resistance of virus.

A number of host cell proteins are incorporated into virions of human immunodeficiency virus type 1 (HIV-1) (Arthur et al., 1992; Franke et al., 1994; Gomez & Hildreth, 1995; Orentas & Hildreth, 1993), and some of them play functional roles. Recently, we and others have shown that HIV-1 grown in lymphoid and myeloid cells incorporates complement control proteins CD46, CD55 and CD59 (Marschang et al., 1995; Montefiori et al., 1994; Saifuddin et al., 1995). The glycosyl phosphatidylinositol (GPI)-anchored proteins CD55 and CD59 are expressed by a variety of cell types including CD4+ T lymphocytes and monocytes (Terstappen et al., 1992). CD55 (decay accelerating factor, DAF) prevents formation of C3 and C5 convertases and accelerates their decay (Fujita et al., 1987), while CD59 (membrane inhibitor of reactive lysis, MIRL) inhibits formation of the cytolytic membrane attack complex (MAC) (Meri, 1994; Rollins & Sims, 1990). The type I integral membrane protein CD46 (membrane cofactor protein, MCP), also expressed by all types of peripheral blood leukocytes including CD4+ T cells (Seya et al., 1988), acts as a cofactor for factor I-mediated cleavage of C3b and C4b (Kojima et al., 1993; Liszewski & Atkinson, 1992). Thus, incorporation of any of these proteins by HIV-1 could augment the complement resistance of the virus.

The capacity of virus-incorporated CD55 and CD59 to mediate complement resistance has been investigated previously (Marschang et al., 1995; Saifuddin et al., 1995). As such, incorporation of both CD55 and CD59 into virions has been shown to enhance complement resistance of HIV-1 (Saifuddin et al., 1995). However, the ability of the individual complement control proteins to induce resistance was not determined. Additionally, the capacity of virion-incorporated CD46 to protect HIV-1 against complement-mediated injury has not yet been investigated. The goal of the current study was to determine whether CD46, CD55 and CD59 could each be incorporated into HIV-1 and contribute to complement resistance. To accomplish this goal, HIV-1 DNA was transfected into control Chinese hamster ovary (CHO) cells or CHO cells expressing human CD46, CD55 or CD59. Subsequently, virus produced from these cells was tested for incorporation of complement control proteins and resistance to complement in the presence of antiviral antibody. The CHO cell line was chosen since virus produced by these cells is moderately sensitive to complement (M. Saifuddin, unpub-

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Expression of human complement control proteins on CHO cells. CHO cells transfected with human CD46, CD55 or CD59 cDNA were incubated with the appropriate specific monoclonal antibodies (b, d, f, respectively). Control CHO cells transfected with vector alone (expressing no human proteins) were also incubated with anti-CD46 (a), anti-CD55 (c) or anti-CD59 (e) antibody. Cells were washed and incubated with goat anti-mouse antibody conjugated with phycoerythrin. After washing, cells were fixed with 1.0% paraformaldehyde and examined by flow cytometry.

Fluorescence Intensity

Fig. 1. Expression of human complement control proteins on CHO cells. CHO cells transfected with human CD46, CD55 or CD59 cDNA were incubated with the appropriate specific monoclonal antibodies (b, d, f, respectively). Control CHO cells transfected with vector alone (expressing no human proteins) were also incubated with anti-CD46 (a), anti-CD55 (c) or anti-CD59 (e) antibody. Cells were washed and incubated with goat anti-mouse antibody conjugated with phycoerythrin. After washing, cells were fixed with 1.0% paraformaldehyde and examined by flow cytometry.

lished observation). Since human cells express several membrane-bound complement control proteins they could not be used for transfection. Protective effect(s) of one complement control proteins on cells would complicate the analysis of lysis experiments.

CHO cells were stably transfected with expression vector pHß/Apr1.neo containing either the human CD46 (MCP-BC1) or CD55 (DAF-4) cDNA clone or expression vector pRC/RSV containing CD59 clone KM32 as described (Lublin et al., 1988; Medof et al., 1987). Cells transfected with expression vector pHß/Apr1.neo containing either the CD46 or CD55 cDNA insert in reverse orientation or expression vector pRC/RSV alone were used as controls. Both CHOCD46 and CHOCD59 cells were cloned by limiting dilution whereas CHOCD55 and CHO control cells were not cloned. All cells were maintained in DMEM (Gibco BRL) containing 10% foetal bovine serum. Cells were tested periodically using the Mycoplasma Detection System (Gen-Probe Inc.) and found to be negative for mycoplasma contamination.

Expression of human CD46, CD55 or CD59 by CHO cells was confirmed by flow cytometry. Single cell suspensions were made from approximately 70% confluent monolayer cultures by treatment with PBS containing 1.0 mM EDTA for 5 min at 37 °C using a cell-scraper (Falcon). Cells (1 × 10⁶) were incubated on ice with 1.0 µg of monoclonal anti-human CD46 (Biodesign International), anti-CD55, anti-CD59 or isotype matched control antibody (Biosource International). After 30 min, cells were washed and incubated for 30 min on ice with phycoerythrin-conjugated goat anti-mouse antibody (Biosource International). After washing, cells were fixed in 1.0% paraformaldehyde and analysed using an Ortho Cytorrone Absolute Flow Cytometer (Ortho Diagnostic Systems Inc.). As shown in Fig. 1 (b, d, f), human CD46, CD55 and CD59 were expressed by appropriately transfected CHO cells while control transfected cells showed background levels of staining (Fig. 1a, c, e, respectively), similar to that obtained with CHO cells expressing human protein stained with an isotype matched control antibody (not shown). Levels expressed on the surface of CHO cells were comparable to those expressed by peripheral blood lymphocytes as determined by flow cytometry (not shown).

Cell-free HIV-1 virions were obtained by transient transfection of each CHO cell type with the full-length, infection-competent HIV-1 clone pNL4-3 (Adachi et al., 1986) (obtained from the AIDS Research and Reagent Program, contributed by Malcom Martin, NIH, Rockville, Md., USA) by a DEAE-dextran method (Saifuddin et al., 1995). At 6 days post-transfection, cell supernatants were collected, filtered (0.45 µm), concentrated by ultracentrifugation through 20% glycerol (Saifuddin et al., 1995) and stored at −70 °C prior to use. The amount of virus in each preparation was determined by p24 ELISA (Coulter).

To determine whether complement control proteins were incorporated into HIV-1 virions, 50 µl aliquots of virus (5 ng p24 antigen/ml) were treated at 37 °C with either fresh serum from an AB⁺ donor or HI serum (dilution 1:5) alone or in combination with rabbit antisera to CD46 (dilution 1:50), CD55 (dilution 1:100) or CD59 (dilution 1:100). The rabbit antisera to CD46 was obtained from CytoMed Inc., and antisera to CD55 and CD59 were prepared as described (Saifuddin et al., 1995). After 60 min, virolysis resulting from antibody-mediated complement activation was quantified by measuring the release of HIV-1 p24 antigen from virus by ELISA as described (Saifuddin et al., 1995). Percent virolysis was calculated by the following formula: [experimental lysis (no detergent) − untreated virus (background)]/[experimental lysis (with detergent) − untreated virus] × 100.

After treatment with complement in the presence of anti-CD46, anti-CD55 or anti-CD59 antisera minimal lysis (1–4%) was observed with virus derived from control CHO cells (pRC/RSV vector transfected cells) expressing no human proteins (Fig. 2a). In contrast, significantly higher levels (P < 0.05, ANOVA) of virolysis (15–54%) were observed with viruses from cells expressing CD46, CD55 or CD59 when incubated with complement plus antibody specific to the human protein expressed by the host cells (Fig. 2a). Anti-CD46, anti-CD55 or anti-CD59 antibody alone or in com-
Protection of HIV by host CD46, CD55 and CD59

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(\textit{a}) (\textit{b})

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\caption{Detection of human CD46, CD55 and CD59 on HIV-1. Cell-free virions were obtained by transient transfection of CHO cell lines expressing either human CD46 (CHO46), CD55 (CHO55) or CD59 (CHO59) or no human proteins (CHO). The control CHO cell line was previously transfected with vector alone and thus expressed no human proteins. (\textit{a}) Concentrated virus was treated with fresh human serum (1:5 dilution) in the presence of rabbit anti-CD46 (1:50 dilution), anti-CD55 (1:100) or anti-CD59 antibody (1:100 dilution). The release of HIV-1 core protein due to virolysis was measured by p24 ELISA, and percent virolysis was calculated as described (Saifuddin \textit{et al.}, 1995) with maximum release of core protein effected by treatment with Triton X-100. Background virolysis (1–2%) caused by complement alone was subtracted from each value of complement plus antibody-mediated lysis. (\textit{b}) Virus preparations were incubated with \textit{Staphylococcus aureus} preloaded with rabbit antiserum to CD46, CD55 and CD59 or normal rabbit serum control. After washing, cells were resuspended in 300 µl 1-0% Triton X-100 and percent virus captured by each antibody was analysed by testing the lysates for p24 antigen. CHO, CHO46, CHO55 and CHO59 HIV-1 preparations containing 2059, 701, 1257 and 522 pg equivalent of p24 antigen, respectively, were used for immunocapture. Results are representative of three experiments and each value represents the mean±SD of multiple determinations in one experiment.}
\end{figure}

bination with HI serum did not induce virolysis (not shown). These results demonstrated that all three proteins were incorporated into HIV-1 virions and were displayed on the virion surface.

An immunocapture assay (Sullivan \textit{et al.}, 1996) using \textit{Staphylococcus aureus} cells was used to confirm whether these proteins are present on HIV-1 preparations. Using specific polyclonal antibodies, 12-5, 14-8 and 18-2% of HIV-1 derived from CHO cells expressing CD46, CD55 or CD59 were captured (Fig. 2\textit{b}) indicating that all three proteins were present on the virions. HIV-1 derived from CHO cells expressing no human proteins was not captured by any of these antibodies. In separate experiments, HIV-1\textsubscript{MN} from human PBMC was assessed by antibody capture assay to compare the level of expression of CD46, CD55 and CD59 with viruses derived from CHO cells expressing human proteins. Relatively lower levels of these proteins (CD46 9-18%, CD55 6-24% and CD59 10-19%) were detected on HIV-1 derived from human PBMC compared to viruses from CHO cells. The levels of incorporation were in agreement with Montefiori \textit{et al.} (1994) who also reported the presence of these proteins on primary isolates of HIV-1 by immunocapture.

To determine whether viruses that incorporated the proteins were more resistant to complement, virus preparations (50 µl) were treated for 60 min at 37 °C with either fresh human serum or HI serum (dilution 1:5) alone or in combination with goat antiserum to HIV-1\textsubscript{RF} gp120 or HIV-1\textsubscript{MN} gp120 (Palker \textit{et al.}, 1988) (obtained through the AIDS Research and Reference Reagent Program, from Thomas Palker, NIH) (dilution 1:20). Virolysis was determined as described above.

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\caption{Complement sensitivity of HIV-1 expressing human complement control proteins. Virus was produced as described in the legend to Fig. 2 and was incubated with fresh human serum in the presence of either anti-HIV-1\textsubscript{RF} or anti-HIV-1\textsubscript{MN} gp120 antibody. Percent virolysis was quantified by measuring the released p24 antigen by ELISA. Results are representative of three experiments and each value represents the mean±SD of multiple determinations in one experiment.}
\end{figure}

Virus derived from control CHO cells was lysed by complement in the presence of antibody against either HIV-1\textsubscript{RF} gp120 (25% lysis) or HIV-1\textsubscript{MN} gp120 (39% lysis) (Fig. 3). Virus derived from the three CHO cell lines expressing CD46, CD55 or CD59 was significantly less sensitive (P < 0.05, ANOVA) to complement-mediated lysis (Fig. 3). The most dramatic reduction in complement sensitivity was observed with virus containing CD59 where only 2% lysis was observed in the presence of anti-gp120\textsubscript{MN} antibody (Fig. 3). Minimal (< 2%) or no lysis was observed with complement alone or HI serum plus anti-gp120 antibody (not shown). These results indicated that human complement control proteins CD46, CD55 and CD59 expressed in CHO cells were incorporated...
into HIV-1 virions and that incorporation of each of these proteins enhanced resistance of virus to complement. Interestingly, the level of expression of these proteins by virions as shown in Fig. 2(a, b) correlated with the level of protection of virions by these proteins as shown in Fig. 3. Thus, the current study indicates that the presence of all three complement control proteins together could efficiently protect HIV-1 from complement in vivo.

This study shows for the first time that each of the proteins CD46, CD55 or CD59 incorporated into HIV-1 virions individually can inhibit complement-mediated virolysis. While previous studies demonstrated that virus derived from host cells expressing CD46, CD55 and CD59 was less sensitive to complement than virus derived from cells expressing only CD46 (Saifuddin et al., 1995), it was not clear which of the proteins (CD55 or CD59) contributed to virus resistance. Further, the capacity of the type I integral membrane protein CD46 to mediate complement resistance of HIV-1 was not previously determined. Thus, while previous studies (Montefiori et al., 1994; Sullivan et al., 1996) showed all three proteins were incorporated it was not determined whether each was incorporated at levels which would provide virus protection. The approach used in this study, which demonstrated functionality of each control protein expressed individually on sensitive virus, was adapted since it provided an advantage over the alternative approach of using inhibitory antibodies directed against individual proteins (e.g. anti-CD46) to inhibit protective function on virus which expresses several of the complement control proteins. For example, antibody inhibition of CD46 function on a virus which expresses high levels of CD59 may not increase virus lysis above 0%, even in the presence of antiviral antibody, since CD59 alone can provide nearly complete resistance in this system (Fig. 3). A previous observation that anti-CD55 did not cause lysis of virus derived from several sources (Saifuddin et al., 1995) could also be explained by the protective effect of CD59 and CD46 on virus.

The complement control proteins CD46, CD55 and CD59 are widely expressed in vivo by cells which can produce HIV-1 such as T lymphocytes, monocytes and macrophages (Terstappen et al., 1992). In spite of their different functional mechanisms each of these proteins has been demonstrated to protect cells from complement-mediated injury when they are individually expressed on cells (Lublin & Coyne, 1991; Zhao et al., 1991). Thus, virus incorporation of these proteins may play an important role in the survival of virus particles in blood and other body fluids that contain complement, and could therefore play a significant role in virus infectivity and pathogenesis of HIV-1 infection.

In summary, this study showed that host cell complement control proteins CD46, CD55 and CD59, are incorporated into HIV-1 virions and that individually each protein enhances the resistance of the virus to complement-mediated lysis. This incorporation may contribute to the in vivo survival of HIV-1 virions in blood and other tissues and thus play a significant role in the virus life-cycle in vivo.

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