Brief Definitive Report

Efficient Destruction of Human Immunodeficiency Virus in Human Serum by Inhibiting the Protective Action of Complement Factor H and Decay Accelerating Factor (DAF, CD55)

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Summary

Activation of the human complement system leads to complement deposition on human immunodeficiency virus (HIV) and HIV-infected cells without causing efficient complement-mediated lysis. Even in the presence of HIV-specific antibodies, only a few particles are destroyed, demonstrating that HIV is intrinsically resistant to human complement. Here we report that, in addition to decay accelerating factor (DAF) being partially responsible, human complement factor H (CFH), a humoral negative regulator of complement activation, is most critical for this resistance. In the presence of HIV-specific antibodies, sera devoid of CFH (total genetic deficiency or normal human serum depleted of CFH by affinity chromatography) lysed free virus and HIV-infected but not uninfected cells. In the presence of CFH, lysis of HIV was only obtained when binding of CFH to gp41 was inhibited by a monoclonal antibody against a main CFH-binding site in gp41. Since CFH is an abundant protein in serum, and high local concentration of CFH can be obtained at the surface of HIV as the result of specific interactions of CFH with the HIV envelope, it is proposed that the resistance of HIV and HIV-infected cells against complement-mediated lysis in vivo is dependent on DAF and CFH and can be overcome by suppressing this protection. Neutralization of HIV may be achieved by antibodies against DAF and, more importantly, antibodies against CFH-binding sites on HIV envelope proteins.

HIV and HIV-infected cells are not lysed by human complement; even in the presence of HIV-specific antibodies, lysis is only partial (1–4). This intrinsic resistance is not due to a failure of HIV surface glycoproteins to interact with complement proteins, since HIV, HIV-infected cells, and purified HIV envelope proteins bind complement proteins with activating functions like C1q or Mannan binding protein (1, 5, 6) and trigger complement fixation. Resistance of HIV to complement could in part be explained by the presence of decay accelerating factor (DAF, CD55) and CD59 on the surface of HIV; covering DAF or CD59 with specific antibodies eliminated their protective effect and resulted in partial lysis of lab strains by normal human serum (NHS) (7, 8). Recently, interaction of complement factor H (CFH), a negative regulator of complement activation, with both envelope glycoproteins of HIV-1, gp120 and gp41, was independently described by Stoiber et al. and Pintér et al. (9–12). CFH down-regulates the amplification loop of complement activation and acts as cofactor for inactivation of C3b to iC3b (13,14). In contrast to C3b, iC3b does not provide further activation of the complement system, and as a consequence, membrane attack complex (MAC) formation does not occur (15). Here we show that resistance to human complement can be completely overcome if DAF and CFH (300–500 μg/ml in NHS) are set out of function.

Materials and Methods

CFH-depleted Human Serum Was Represented by Genetically CFH-deficient Serum or Prepared from NHS. Lyophilized and titrated human plasma (S-1767; Sigma Chemical Co., St. Louis, MO) was dissolved in ice-cold ultrapure water and depleted of CFH by incubation with CNBr-activated Sepharose (Pharmacia, Uppsala, Sweden) coupled with the mAb 5H5, specific for human CFH (9). Uncoupled Sepharose beads were used as a control to show that depletion was due to the antibody and not to unspecific adsorption to Sepharose. The amount of Sepharose-bound mAb was used in a sixfold excess as compared with the calculated amount of CFH present in the plasma sample. Incubation was performed at 4°C for 2 h. CFH concentration was checked after

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sample collection in ELISA and SDS-PAGE, followed by Western blot. All Sepharose supernatants were filter sterilized before use in cell assays.

Purification of Antibodies from Sera of Patients with AIDS and NHS. IgG from sera of AIDS patients and from NHS were purified from the pass-through of a DEAE Affi-Gel Blue column (BioRad, Richmond, CA) and vacuum ulrafiltration on membranes (Micro Prodicon; Spectrum, Houston, TX) with a 100-kD cut-off, to eliminate transferrin. The AIDS sera used as a source of human AIDS Ig were chosen on the basis of their reactivity in ELISA assays against gp120 and gp41 (American Biotechnologies, Cambridge, MA). Normal sera were controlled for the absence of anti-env antibodies with the same method.

Assay of Complement-dependent Cytotoxicity (CDC). Each experiment contained 10^5 of HIV-infected (8E5) and, as control, noninfected CEM (American Type Culture Collection CCL119) cells in protein-free medium and four different doses of NHS depleted of CFH (NHS^{CFH-}) as complement source (1:10, 1:20, 1:40, 1:80, and no complement added). Before being exposed to complement, HIV-infected and uninfected cells were incubated with 50 μg/ml of purified human antibodies from either HIV^+ or healthy donors for 1 h on ice. The cells were washed and resuspended in duplicates in prediluted serum samples. After 1 h at 37°C, the reaction was stopped by the addition of 2.5 mM EGTA (final concentration). Cell viability was monitored by trypan blue exclusion and calculated on the basis of the following formula: % CDC = (number of dead cells/number of dead cells + live cells) × 100.

Complement-mediated Lysis of HIV. NHS or serum from a patient with genetic CFH deficiency (CFH^{def}) (16) (both end dilutions 1:10) was incubated with purified antibodies isolated from HIV^+ (Ig anti-HIV) on ice. In some cases, polyclonal antibodies against DAF (anti-DAF) were added (end dilution 1:100). The solutions were transferred to an ELISA plate, which was precoated with antibodies against p24. Constant amounts of primary isolates (92RW021, 92TH024, IBK-0295) and the lab strain HIV-IIIB were added, and the mixture was incubated for 1 h at 37°C to allow complement-mediated lysis of viral particles and release of p24 in the absence of detergent. Free p24 in the supernatant, an indicator for lysis and neutralization of HIV (8, 17), bound to the antibody on the ELISA plate. After four washing cycles with PBS without detergent, a second antibody against p24 was added, followed by two further washing steps with PBS and two with PBS, containing 0.1% Tween 20. The ELISA was developed with biotin-streptavidin, and the optical density of the samples (OD_{sample}) was measured. NP-40 was used to determine 100% lysis (OD_{NP40}) and the background (OD_{back}) by incubation of HIV with NHS without Ig anti-HIV. The percentage of lysis was calculated by the following formula: $(OD_{sample}-OD_{back}/OD_{NP40}-OD_{back}) \times 100$. The experiments were performed in duplicate.

Results and Discussion

To test the hypothesis that direct binding of CFH to the envelope of HIV-1 might protect the virus from destruction by human serum, 8E5 cells, chronically infected with HIV (18), and noninfected CEM cells were pretreated with Ig obtained from HIV^+ or healthy individuals and exposed to NHS^{CFH-}. As shown in Fig. 1, only HIV-infected cells were susceptible to CDC. Furthermore, CDC was strictly dependent on the use of NHS^{CFH-}; application of NHS instead of NHS^{CFH-} had no destructive effect. To prove formally the protective role of CFH, NHS^{CFH-} was reconstituted with increasing amounts of CFH. 50% protection of HIV-infected cells could be obtained by the addition of ~8 μg CFH/ml (Fig. 2), corresponding to a reconstitution of ~5% of the normal CFH concentration in plasma.

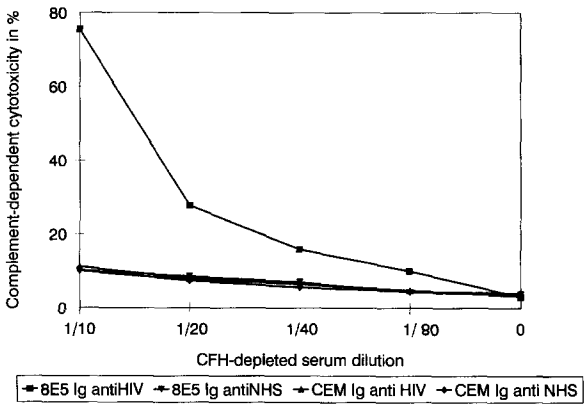

Figure 1. Assay of CDC. HIV^+ (8E5) and uninfected cells (CEM) were incubated with CFH-depleted serum containing purified Ig from HIV^+ sera (Ig anti-HIV) or Ig from NHS, and CDC was determined.

In the next set of experiments, free viral particles from different primary isolates, 92RW021, 92TH024, IBK-0295, and lab strain HIV-IIIB, were incubated with NHS or NHS^{CFH-}, each in combination with different antibodies. In accordance with Spear et al. (4), in the presence of HIV-specific antibodies and NHS complement–dependent virolysis, up to 30% lysis in the case of HIV-IIIB was obtained. Primary isolates were more resistant to lysis, that is, only 12% lysis was obtained with IBK-0295, a virus isolated from an Innsbruck patient and passaged only once for these experiments. Therefore, 88% of this isolate was unaffected by human complement (Fig. 3). Lysis could be considerably increased by the presence of an antibody against DAF, eliminating the DAF-mediated anticomplement effect. Incubation of various HIV strains with Ig anti-HIV

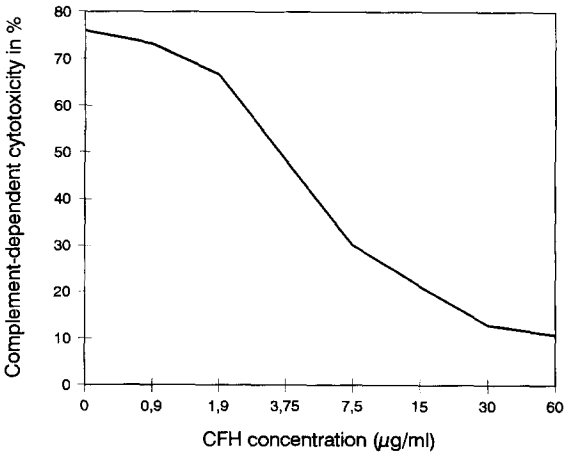

Figure 2. Effect of addition of CFH to CFH-depleted serum. The CDC decreased as increasing amounts of CFH were added. The percentages of CDC were determined as described in Materials and Methods.

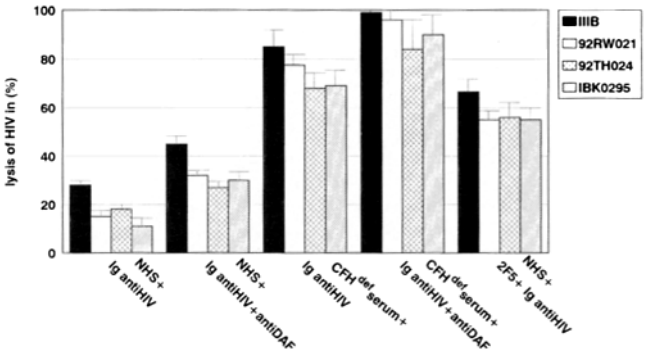

Figure 3. Lysis of cell-free HIV by human serum. Different HIV isolates (the lab strain HIV-IIIB and three primary isolates) were incubated with NHS or serum from a patient with total CFH^{def} in the presence of purified IgG from sera of HIV^+ individuals (Ig anti-HIV). In some assays, anti-DAF antibodies were added as indicated. The percentage of complement-mediated lysis was monitored by measuring free p24 in the supernatant as described in Materials and Methods.

and serum derived from a patient with total CFH^{def} resulted in release of 85% of total p24, indicating a very efficient inactivation of HIV. These findings clearly demonstrate that CFH is the main contributor to resistance of HIV against lysis by human complement. Interestingly, a comparable defense strategy was shown for streptococci, which bind CFH on their surface protein M (19). Combining CFH^{def} and anti-DAF in the presence of Ig anti-HIV, nearly all free HIV particles were destroyed through the lytic action of human complement (Fig. 3). To prove directly whether binding of CFH to gp41 was critical for CFH-mediated protection, the different HIV strains were tested in the presence of mAb 2F5. This antibody covers the ELDKWAS motif in gp41 (aa 665–671 in HIV-IIIB) and is known to neutralize HIV in vitro (17); in addition, this site in gp41 was described as a binding region for factor H (8–11). As shown in Fig. 3, incubation of HIV with 2F5 and addition of NHS in combination with Ig anti-HIV lead to the destruction of ~60% of available HIV. Obviously, the CFH-mediated protection of HIV from complement-dependent lysis was abrogated because of interference by mAb 2F5 with CFH binding to the ELDKWAS motif of the viral envelope protein.

These results suggest that DAF as part of the viral membrane and exogenous CFH secondarily attached to the HIV envelope proteins together are sufficient to explain the efficient, intrinsic resistance of HIV to human complement. Overcoming these two barriers should be appropriate goals for new vaccine strategies. By activating complement, HIV coats itself with C3, thus enhancing its infectivity and providing, for example, for follicular localization (20–24) while avoiding complement-mediated destruction via complement control proteins DAF and CFH. This mechanism might represent a general strategy also used by other complement-resistant human retroviruses.

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