Human Immunodeficiency Virus Type 1 Activates the Classical Pathway of Complement by Direct C1 Binding through Specific Sites in the Transmembrane Glycoprotein gp41

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Summary

Human immunodeficiency virus type 1 (HIV-1), in contrast to animal retroviruses such as murine leukemia virus, is not lysed by human complement. Nevertheless, HIV-1 activates complement via the classical pathway independent of antibody, and C3b deposition facilitates infection of complement receptor-bearing cells. Using gel exclusion chromatography on Sephacryl S-1000, purified virions were found to bind 125I-labeled Clq, but not 125I-labeled dimeric proenzyme Cls. Virions activated the C1 complex, reconstituted from Clq, proenzyme Clr, and 125I-labeled proenzyme Cls, to an extent comparable with that obtained with immunoglobulin G–ovalbumin immune complexes. To determine the activating viral component, recombinant viral proteins were used: in the solid phase, soluble gp41 (sgp41) (the outer membrane part of gp41, residues 539–684 of gp160) bound Clq, but not dimeric proenzyme Cls, while gp120 was ineffective. In the fluid phase, sgp41 activated the C1 complex in a dose- and time-dependent manner, more efficiently than aggregated Ig, but less efficiently than immune complexes. To localize the C1 activating site(s) in gp41, synthetic peptides (15-residue oligomers spanning amino acids 531–695 of gp160) were used. Peptides covering positions 591–605 and 601–620 and, to a lesser extent, positions 561–575, had both the ability to bind Clq and to induce C3 deposition. These data provide the first experimental evidence of a direct interaction between the C1 complex and HIV-1, and indicate that C1 binding and activation are mediated by specific sites in gp41.

Retroviruses isolated from avian, feline, murine, and simian sources have been found to be lysed by normal human serum (1–3). Lysis is induced by direct antibody-independent triggering of the classical complement pathway (4). It was previously believed that this mechanism protected the individual from retroviral disease (2). Human retroviral pathogens (HIV-1 and HTLV-1) have since been identified, and several laboratories have shown that these viruses are not lysed efficiently by human serum (5, 6), although animal sera from felidae or muridae are capable of lysis (7).

As in the case with other retroviruses, cells infected with HIV-1 activate the complement system independent of antibody via the alternative pathway. Subsequent deposition of C3b/C3d resulted in rosetting between HIV-1-infected cells and cells bearing complement receptors (CR) (8). In contrast, isolated HIV-1 (strain IIIB) activated the classical pathway independent of antibody (8). The biological relevance of the latter mechanism was demonstrated through the observation that infection of CR-bearing cells by HIV-1 is enhanced at low multiplicity of infection (9, 10), i.e., under conditions that probably represent the typical in vivo situation during the first contact between HIV-1 and the host. The validity of this concept has been proven by other groups (11–13).

The fact that complement activation by HIV-1 does not result in lysis of the virus could be explained either by a viral component interfering with the complement cascade or by restriction mechanisms similar to those protecting cells of an individual against its own complement system (14, 15), such as decay-accelerating factor (16) and membrane cofactor protein (17); such a factor originating from the host cell may be embedded in the membrane of HIV-1 and protect the retrovirus against the lytic activity of human complement. Among examples of such a protection mechanism in other viruses is glycoprotein C of HSV-1, which binds C3b (18); vaccinia virus has a protein with structural homology to C4b-binding protein (19) and EBV accelerates the decay of the alternative pathway C3 convertase (20, 21).

In an attempt to elucidate the molecular mechanisms in-
volved in the early steps of the activation of the classical pathway of complement by HIV-1, we provide the first experimental evidence of a direct interaction between the C1 complex and HIV-1 and show that C1 binding and activation are mediated by specific sites in gp41.

Materials and Methods

Reagents and Buffers. IgG-OVA immune complexes were prepared at equivalence as described previously (22). Heat-aggregated IgG were prepared by heating purified rabbit Ig (15 mg/ml) for 15 min at 63°C. Particulate material was removed by centrifugation and the soluble aggregates were used for C1 activation.

Veronal-buffered saline (VBS)1 contained 5 mM sodium barbital (pH 7.4), 0.15 mM CaCl2, 1 mM MgCl2, and either 150 mM NaCl (physiological ionic strength) or 75 mM NaCl (half-physiological ionic strength). Recombinant protein p138 from EBV was obtained from Biotech (Dreieich, Germany).

Cl Subcomponents. Clq, proenzyme Clr, and proenzyme Cls were isolated from human plasma as described previously (22, 23). The concentrations of purified Clq, Clr, and Cls were determined by using values of E (1%, 1 cm) at 280 nm of 6.8, 12.4, and 14.5, and molecular weights of 459,300, 86,300, and 78,900 (24, 25). Clq and Cls were labeled with 121I either by the immobilized lactoperoxidase-glucose oxidase method using Enzymobeads (Bio-Rad Laboratories, Richmond, CA) or with iodobeads (Pierce Chemical Co., Rockford, IL) as recommended by the manufacturers. Unbound 121I was removed by exhaustive dialysis or by centrifugation on a Sephadex G-50 fine column (Pharmacia Fine Chemicals, Uppsala, Sweden) as described previously (26).

Cells and Virions Preparation. H9 cells chronically infected with HIV-1 (HTLV-IIIB strain) were cultivated with four parts of uninfected H9 cells in RPMI 1640 supplemented with 10% FCS, 2 mM glutamine, and antibiotics (all from Seralab, Sussex, UK) for 48–72 h. Supernatants were harvested by centrifugation at 400 g for 10 min, followed by centrifugation at 800 g for 30 min, and subsequent filtration through 0.2-μm membrane (Millipore Continental Water Systems, Molsheim, France). These supernatants were centrifuged at 100,000 g for 90 min to concentrate the virus. Virus was resuspended in 500 μl of VBS at half-physiological ionic strength and loaded under containment conditions onto a disposable 10-ml Sephacryl-S-100 column (Pharmacia Fine Chemicals) equilibrated with the same buffer. Virions were eluted with this buffer and fractions were collected. P24 was measured with a capture ELISA (Coulter Immunology, Hialeah, FL) using recombinant p24 as a standard (27). In parallel, supernatants of uninfected H9 cells were centrifuged, passed through the column, and used as a control.

Proteins and Peptides from HIV-1. Recombinant protein gp120 (HTLV-IIIB strain) was obtained from the MRC AIDS Directed Program (Herts, UK). Soluble gp41 (sgp41), the proposed outer membrane part of gp41 (28), was derived from clone BH 10. The membrane part of gp41 (28), was derived from clone BH 10. The protein was separated from the rest of the virions by centrifugation at 100,000 g for 90 rain to concentrate the virus. Virus was resuspended in 500 μl of VBS at half-physiological ionic strength. Recombinant protein p138 from EBV was obtained from Biotech (Dreieich, Germany).

Results

Clq, but Not Cls, Binds to HIV-1. Since classical pathway activation by HIV-1 has been proven (8), we wanted to determine which

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1 Abbreviation used in this paper: VBS, veronal-buffered saline.
Figure 1. C1q (C1s) binding to HIV-1. $^{125}$I-labeled C1q (A) or C1s (B) were incubated with HIV-1 and then subjected to gel exclusion chromatography on Sephacryl S-1000. [A and B] $^{125}$I; (O and ●) p24; (closed symbols and continuous lines) samples containing HIV-1; (open symbols and dotted lines) samples containing control preparation.
dependent manner, less efficiently than IgG-OVA immune complexes, but more efficiently than heat-aggregated Ig (Fig. 4). In contrast to sgp41, gp120 had no significant effect on C1 activation within the range tested (Fig. 4). Considering that recombinant proteins obtained from bacterial expression systems are occasionally contaminated by LPS, a known activator of the C1 complex (35), it appeared necessary to verify that the activating effect observed with sgp41 was not due to trace amounts of LPS. To this end, control experiments were performed in the presence of varying concentrations of polymixin B, an antibiotic that binds to LPS and thereby abrogates its C1-activating ability (35). As shown in Table 1, polymixin B only had a slight inhibitory effect on C1 activation by sgp41, comparable to that observed in the case of IgG-OVA immune complexes. This effect likely reflected an inhibition of the intrinsic C1 activation mechanism, probably due to C1 dissociation, as spontaneous C1 activation (in the absence of activator) was significantly slowed down by polymixin B, as illustrated in Table 1. This hypothesis was further supported by kinetic experiments (data not shown). In contrast, activation of C1 by p138, a recombinant protein from EBV, was abolished in the presence of 1 mg/ml polymixin B, indicating that this preparation was likely contaminated by LPS.

Kinetic experiments performed in the presence of polymixin B indicated that sgp41 and IgG-OVA immune complexes both induced a marked increase in the rate of C1 activation, resulting in

<table>
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<th>Polymixin B</th>
<th>C1 + buffer</th>
<th>C1 + sgp41</th>
<th>C1 + IgG-OVA complexes</th>
<th>C1 + p138</th>
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<td>mg/ml</td>
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<td>3.6</td>
<td>54.4</td>
<td>61.5</td>
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C1 activation was measured after incubation of the reconstituted complex for 20 min at 30°C with different activators (sgp41, 42 μg; IgG-OVA complexes, 40 μg; p138, 42 μg), in the presence of varying concentrations of polymixin B.

Figure 2. Kinetics of C1 activation by HIV-1 and IgG-OVA immune complexes. The reconstituted C1 complex (1 μg) was incubated for varying periods at 30°C with (□) Ig-OVA immune complexes (1 μg), (■) HIV-1 (30 ng of p24), and (▲) control preparation.

Figure 3. C1q binding to IgG-OVA immune complexes and recombinant proteins from HIV-1. Serial dilutions of IgG-OVA immune complexes, recombinant proteins, and control protein (BSA) were bound to ELISA plates, and subsequent binding of 125I-labeled C1q was measured. (■) IgG-OVA immune complexes, (○) sgp41, (●) gp120, (□) BSA.

Figure 4. C1 activation in the presence of varying amounts of IgG-OVA immune complexes, aggregated Ig, and recombinant proteins from HIV-1. C1 activation was measured after incubation of the reconstituted complex (12 μg) for 20 min at 30°C with (□) Ig-G-OVA complexes; (●) sgp41; (■) heat-aggregated Ig; (▲) gp120.

Figure 5. Kinetics of C1 activation by IgG-OVA immune complexes and recombinant sgp41. The reconstituted C1 complex (12 μg) was incubated for varying periods at 30°C in the presence of 1 mg/ml polymixin B, (■) C1 alone; (□) C1 + Ig-G-OVA complexes (28 μg); (●) C1 + sgp41 (28 μg).
Figure 6. Localization of the C1 activating sequence in gp41. Synthetic peptides (1 μg per well) were dried onto ELISA plates. Subsequent binding of 125I-labeled Clq (A) and C3 deposition (B) were measured. Peptides are numbered according to reference 29. IC, BSA, control measurements obtained with IgG-OVA immune complexes (1 μg), and BSA (1 μg).

Discussion

This paper analyzes the molecular basis of the antibody-independent activation of the classical pathway of complement by HIV-1 and provides the first experimental evidence of: (a) a direct interaction between the virus and the C1 complex and (b) the ability of the virus to activate the reconstituted C1 complex. These experiments are probably representing the typical in vivo situation during the first encounter between HIV-1 and the host. In this first phase of infection, HIV-1 could be targeted through antibody-independent complement activation to complement receptor-positive cells such as monocytes/macrophages (10). The earlier suggestion that HIV-1 activates the classical pathway (8) is clearly supported by the direct binding of Clq and activation of the C1 complex. These results are further confirmed by the experimental data obtained with recombinant proteins and synthetic peptides. The fact that the alternative pathway was shown to be involved, as stated in a recent report (12), remains unclear. We do not want to rule out the possibility that the alternative pathway may also be involved in the mechanism described here, since C3 deposition via the classical pathway may lead to subsequent activation of the alternative pathway.

Our results with the human retrovirus are in agreement with previous studies indicating direct triggering of the classical pathway by animal retroviruses (4). In the case of MuLV (the best investigated example), direct attachment of the C1 complex to the viral surface was also demonstrated (36). However, in contrast to HIV-1, both Clq and C1s were shown to bind to the viral surface (36).

To define the viral component responsible for the interaction with the C1 complex, we used recombinant sgp41 (representing the proposed outer membrane part of gp41 [28]) and recombinant gp120. Two different assays (Clq binding and C1 activation) gave similar results. Sgp41 was shown to interact with Clq and to induce C1 activation, whereas gp120...
was ineffective in both tests. The rather weak affinity observed in the solid phase binding assays may be explained by the facts that sgp41 is probably monomeric and that the C1q binding affinity is enhanced by multivalent interactions (37). On the other hand, the oligomeric state of gp41 on intact virions (38, 39) probably favors a multivalent binding of C1q and thereby induces rapid activation of the C1 complex. This hypothesis is further supported by the observation that exhaustive removal of SDS from the sgp41 preparation both induces aggregation of the protein and enhances its activation potential (data not shown). The observed binding of C1q to sgp41 is reminiscent of previous studies on MuLV, where the C1 binding component was identified as p15e, the transmembrane protein of MuLV (40).

The peptide studies demonstrated two potential C1 binding sites in gp41. The major site (amino acids 591–620) (Fig. 7 B) includes both the immunodominant (41) and the putative immunosuppressive regions (42) of gp41; both regions are highly conserved among most retroviruses (43). The second site (amino acids 561–575) was less efficient in both C1q binding and C3 deposition assays. This site is probably part of the region involved in the interaction between gp120 and gp41 (44–46). It should be stressed that none of these sequences contain the ExKxK motif, previously defined as the binding site for C1q on the Cγ2 domain of IgG (47).

Interestingly, there is recent evidence that the epitope 591–620, which contains the major C1 binding and activating sequence, is exposed after sCD4 binding to gp120 (48). This change of the steric configuration of the CD4-gp120-gp41 complex could facilitate the interaction between gp41 and the C1 complex. The mechanisms described here probably represent the molecular basis for the complement-dependent enhancement of HIV-1 infection (9–13), which presupposes complement activation independent of antibody. Different of this strictly complement-dependent enhancement is the proposal of Robinson and Mitchell (49), who showed that, in addition to an exclusively antibody-dependent enhancement (Fc receptor-mediated antibody-dependent enhancement) (50–53), a mechanism exists in which complement facilitates the antibody-dependent enhancement of infection (complement-mediated antibody-dependent enhancement) (11, 13, 54–59). Besides its enhancing effect on HIV-1 infection, the addition of complement also reduced or abrogated the HIV-1-neutralizing activity of antibodies (54, 60). Interestingly, Robinson et al. (61, 62) mapped complement-mediated antibody-dependent enhancement to a synthetic peptide (amino acid residues 586–620), which in our view contains the C1 activating domain. The common feature between our and Robinson's concepts is the role of human complement. Clearly, we stress the importance of complement in the early phase.

This report provides experimental evidence of a direct interaction between the C1 complex and HIV-1, and indicates that C1 binding and activation are mediated by specific sites in gp41. We suspect that this fact is of major importance in the early phase of the infection by HIV-1.

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