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Complement Mediates the Binding of HIV to Erythrocytes¹

Eliska Horakova,* Olivier Gasser,* Salima Sadallah,* Jameel M. Inal,* Guillaume Bourgeois,* Ingrid Ziekau,† Thomas Klimkait,† and Jürg A. Schifferli^{2*}

A fraction of HIV is associated with erythrocytes even when the virus becomes undetectable in plasma under antiretroviral therapy. The aim of the present work was to further characterize this association in vitro. We developed an in vitro model to study the factors involved in the adherence of HIV-1 to erythrocytes. Radiolabeled HIV-1 (HIV) and preformed HIV-1/anti-HIV immune complexes (HIV-IC) were opsonized in various human sera, purified using sucrose density gradient ultracentrifugation, and incubated with human erythrocytes. We observed that, when opsonized in normal human serum, not only HIV-IC, but also HIV, bound to erythrocytes, although the adherence of HIV was lower than that of HIV-IC. The adherence was abolished when the complement system was blocked, but was maintained in hypogammaglobulinemic sera. Complement-deficient sera indicated that both pathways of complement were important for optimal adherence. No adherence was seen in C1q-deficient serum, and the adherence of HIV was reduced when the alternative pathway was blocked using anti-factor D Abs. The adherence could be inhibited by an mAb against complement receptor 1. At supraphysiological concentrations, purified C1q mediated the binding of a small fraction of HIV and HIV-IC to erythrocytes. In conclusion, HIV-IC bound to erythrocytes as other types of IC do when exposed to complement. Of particular interest was that HIV alone bound also to erythrocytes in a complement/complement receptor 1-dependent manner. Thus, erythrocytes may not only deliver HIV-IC to organs susceptible to infection, but free HIV as well. This may play a crucial role in the progression of the primary infection. *The Journal of Immunology*, 2004, 173: 4236–4241.

Human immunodeficiency virus infects and replicates in different cell types in the body. These cells are a source of ongoing virus release into the bloodstream, and they form HIV reservoirs even under highly active antiretroviral therapy (1–3). Measurements of the plasma load of HIV represent a fairly good marker for the ongoing production of HIV. However, infectious HIV is found not only in plasma, but also attached to many circulating cells, such as neutrophils, B lymphocytes, platelets, and, as recently demonstrated, erythrocytes (4–7). Interestingly, although the plasma load of HIV diminishes to levels below the limit of detection under highly active antiretroviral therapy, the virus remains present on erythrocytes (100–1000 copies of HIV-1 RNA on erythrocytes in 1 ml of whole blood) (6). This remaining blood pool of HIV very likely reflects the continuous replication in infected cells, with subsequent release and attachment to erythrocytes. Because HIV on erythrocytes remains infectious, this pool of viruses might contribute to continuous reinfection of other cells.

The mechanism by which HIV is attached to erythrocytes in vivo is unknown. Whereas many cells have specific receptors for HIV, none is known on erythrocytes. Except during the primary infection, HIV is unlikely to be free in the circulation, because most patients produce specific Abs in the weeks following primary

infection. Immune complexes (IC)³ formed by HIV/anti-HIV Abs (HIV-IC) are present in plasma and can bind specific FcRs (8, 9). Complement activation followed by C3 fragment deposition on the HIV-IC allows efficient binding of HIV to cells such as B lymphocytes, which have receptors for C3 fragments, in particular CD35 and CD21 (10–12). CD35, also called complement receptor 1 (CR1), binds C3b and C3bi and is expressed on erythrocytes as well. Although the number of CR1 per erythrocyte is low (~300–400), its clustered distribution allows an efficient adherence of opsonized bacteria or IC (13, 14), a process described as immune adherence (15, 16). Experiments in humans have shown that different Ag/Ab IC bind to erythrocytes and are transported to liver and spleen (17, 18). Whereas IC reaching the liver are rapidly degraded, those fixed in the spleen appear to remain unaltered for days, thus allowing Ags to be exposed to the immune response machinery (19).

The present work was undertaken to better define the binding of HIV-1 to erythrocytes in vitro using a model of HIV-IC and HIV-1 as a control. The hypothesis was that HIV-1 binding to erythrocytes was dependent on the presence of specific Abs with formation of HIV-IC, followed by complement activation and C3b binding, and finally immune adherence to CR1. The results indicated that HIV-IC behaved like other IC and could adhere to erythrocytes, but the main finding was that HIV-1 virus particles in the absence of specific Abs were also capable of adhering to erythrocytes in the presence of complement.

Materials and Methods

Virus purification and radiolabeling

HIV used in all experiments was of HIV-1 type IIIB of the North American isolate, cultured on Hut/4–3 cells, a cell line derived from human lymphocytes. Cell supernatants containing the virions were inactivated by incubation for 1 h at 62°C in the presence of 0.02% formaldehyde to preserve

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³ Abbreviations used in this paper: IC, immune complex; CR1, complement receptor 1; HIV-IC, immune complexes formed by HIV/anti-HIV Abs; HIV-IC*, HIV*/anti-HIV immune complexes; IV*, ¹²⁵I-labeled HIV-1; MBL, mannose-binding lectin; NHS, normal human serum; SDGU, sucrose density gradient ultracentrifugation.

the antigenic properties of the virions (20). Supernatants were separated from cell debris by low speed centrifugation at $400 \times g$ for 15 min and passed through a $0.45\text{-}\mu\text{m}$ pore size membrane filter (Minisart filter; Sartorius, Göttingen, Germany). The viral suspension was further concentrated using Centriprep centrifugal filter devices (M_r 10,000 cut-off; Millipore, Bedford, MA), the concentrate was ultracentrifuged twice for 1 h each time at $160,000 \times g$, and the pellet was resuspended in PBS. HIV-1 was then radiolabeled with ^{125}I using Iodogen (Bio-Rad, Richmond, CA). ^{125}I -Labeled HIV-1 (HIV*) was purified by gel filtration on a Sephadex G-25 column (Pharmacia Biotech, Uppsala, Sweden). HIV* was stored at -80°C until use.

The preservation of antigenic epitopes was demonstrated by Western blotting. Inactivated HIV-1 concentrate was separated by SDS-PAGE under reducing conditions, blotted onto nitrocellulose, and probed with human polyclonal anti-HIV-Ig as primary Ab. Bound Abs were detected using a combination of mouse biotinylated anti-human IgG (Southern Biotechnology Associates, Birmingham, AL) and HRP-coupled streptavidin. The final detection was by ECL (Amersham Biosciences, Arlington Heights, IL) with BioMax films (Eastman Kodak, Rochester, NY).

Sera and Abs

The normal human sera (NHS) used as complement source were obtained from healthy individuals with blood group O, seronegative for HIV-1 and HIV-2. Heat inactivation, i.e., destruction of complement activity, was achieved by incubating the serum for 30 min at 56°C . Sera deficient in complement factors C1q and C2 were donated by patients with inherited complement deficiencies. To restore the full activity of complement, as assessed by hemolytic assays, purified human factor C1q (Advanced Research Technologies, Boston, MA) and purified human factor C2 (Calbiochem, Bad Soden, Germany) were added at physiological concentrations. Two hypogammaglobulinemic sera were donated by Prof. A. Fasth (Department of Pediatrics, University of Göteborg, Göteborg, Sweden) and Prof. D. Webster (Royal Free Medical School, University College London, London, U.K.). A reduced complement activity was demonstrated (complement hemolytic activity, $\sim 50\%$ of the normal control), probably due to storage and transportation. No other complement function tests could be performed due to the small volumes of the serum samples. A complete and specific inhibition of the alternative pathway of complement was achieved by adding rabbit polyclonal anti-factor D IgG Abs to NHS (21), as assessed by complement hemolytic activity and alternative pathway hemolytic activity assays. Hemolytic assays were performed as described previously (22). Other sera used were NHS supplemented with either 10 mM EDTA or 5 mM MgCl and 10 mM EGTA.

Polyclonal human anti-HIV Ig purified from sera of HIV-1-infected individuals (HIV-IG, catalogue no. 3957) was donated by the National Institutes of Health AIDS Research and Reference Reagent Program. Normal human Ig was isolated from plasma of a healthy donor seronegative for HIV-1 and HIV-2 using protein G chromatography. C3b/C4b binding sites on erythrocyte CR1 were blocked using mAbs 3D9 and J3D3 (23).

Erythrocytes

Human erythrocytes were purified from fresh blood of healthy volunteers with blood group O using dextran sedimentation. Briefly, the blood was centrifuged at $680 \times g$ for 7 min, and plasma and buffy coat were removed. Remaining blood cells were mixed with 4% dextran and PBS and left on ice for 40 min. After sedimentation, the supernatant was removed with the upper layer of the erythrocytes, and the rest of the purified RBC were washed five times with RPMI 1640 medium (Life Technologies, Basel, Switzerland), each time removing the upper layer of the cells with the supernatant. The final contamination with leukocytes was reduced to ≤ 1 leukocyte/10,000 RBC. For all binding assays, freshly purified erythrocytes were used or, when indicated, they were preincubated 15 min at room temperature with CR1-blocking mAbs 3D9 and J3D3 in a 400-fold excess of Ab over the estimated number of CR1 molecules on the surface of erythrocytes. Sheep erythrocytes were used as a control (Dade Behring, Paris, France).

IC formation and purification

HIV*/anti-HIV-IC* were formed by incubating $\sim 1.5 \mu\text{g}$ of HIV* with $85 \mu\text{g}$ of anti-HIV Ig in a total volume of $220 \mu\text{l}$ in a shaking water bath for 60 min at 37°C . IC opsonization with complement was achieved by incubation with either fresh NHS (dilution, 1/3) or other complement-deficient sera for 7 min at 37°C . The opsonized HIV-IC* were purified by sucrose density gradient ultracentrifugation, as previously described (17, 24–26). In short, sucrose gradients consisted of five layers of sucrose solutions 50 to 10% in PBS. Opsonized HIV-IC* or HIV* were overlaid on the gradients and ultracentrifuged for 2.5 h at $116,000 \times g$. Fractions of $200 \mu\text{l}$ were

taken from the bottom of the tube, and the cpm of each fraction were determined. Fractions containing purified opsonized HIV* or HIV-IC*, located near the bottom of the gradient, were then tested for their capacity to bind to erythrocytes.

Binding assays

Purified opsonized HIV-IC* or HIV* were incubated with erythrocytes at a ratio of 6000 cpm of the opsonized HIV-IC* or HIV*/ 2×10^8 erythrocytes for 10 min at 37°C . The experimental conditions were established to correspond to the situation in vivo. In vivo, the number of viruses are up to 100,000 copies/ml blood, i.e., one virus for 10^4 erythrocytes. Based on the specific activity 1.2×10^4 cpm/ μg radiolabeled viral concentrate derived from PCR and Bradford protein assay data of our HIV stock solution, we estimated that there was about one virion per 7500 erythrocytes in the binding reaction. In the initial experiments, identical binding results were obtained using 3-fold lower or higher erythrocyte concentrations, indicating that the amount of erythrocytes was not limiting in our standard experiments. After incubation, the reaction was stopped by adding ice-cold RPMI 1640 and was centrifuged for 3 min at $170 \times g$. A fraction corresponding to half the final volume was removed, and the pellet of erythrocytes was resuspended in the remaining half. Then, both fractions were measured in a gamma counter, and the percentage of HIV-IC* or HIV* bound to erythrocytes was calculated. All measurements were made in duplicate unless otherwise stated. Statistical analysis was performed using the two-sample *t* test.

Results

IC model

We first determined by Western blotting whether the inactivated viral particles were still recognized by polyclonal anti-HIV Abs. The Western blot revealed several bands with the same m.w. as untreated viral proteins, indicating that the antigenic properties of the virions were preserved (Fig. 1*a*).

The radiolabeled and opsonized viral particles or ICs were purified by sucrose density gradient ultracentrifugation (SDGU). Analysis of the different gradient fractions showed that only part of the ^{125}I was incorporated into high density particles (fractions 4–6) corresponding to HIV, although much of the ^{125}I bound to proteins or aggregates of 19 S or less (19S = fraction 12 corresponding to IgM; Fig. 1*b*). The addition of specific Abs produced a shift to higher density fractions as expected for the formation of HIV-IC*. We then analyzed the binding of the different fractions collected from the sucrose density gradient to erythrocytes. In the absence of opsonization with NHS, none of the fractions bound to

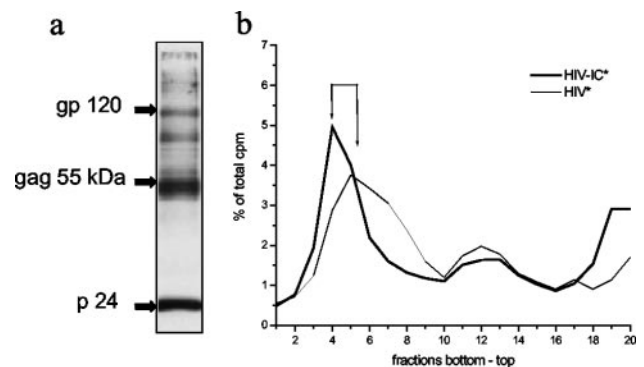


FIGURE 1. *a*, Western blot showing the recognition of unaltered HIV protein epitopes after formaldehyde inactivation of HIV. *b*, Sucrose density gradient purification of HIV* and HIV-IC*. HIV* and HIV-IC* opsonized with NHS were separated from soluble proteins and free radioactivity by sucrose density gradient ultracentrifugation. Complete viral particles were found in the bottom fractions 4–6. The arrows mark the shift toward the bottom between HIV* and HIV-IC* due to the higher density of the HIV-IC*. Protein aggregates did not pass further than fraction 12; soluble proteins and free radioactivity appear at fraction 19.

erythrocytes ($\leq 5\%$). When pre-exposed to NHS, most virion particles/Ab complexes (fractions 4–6) bound to erythrocytes ($>60\%$). The other fractions bound less or not at all ($\leq 10\%$; data not shown). Based on these results, additional assays were conducted using exclusively virion particles isolated from the pooled fractions 4 and 5.

Binding of HIV-IC* to erythrocytes

As shown in Fig. 2a, opsonization by complement was crucial for the binding of HIV-IC*. HIV-IC* opsonized in NHS as a source of complement bound strongly (65–75%). In contrast, HIV-IC* showed no binding when NHS was heated for 30 min at 56°C, which inactivates both pathways of complement. Similarly, we observed no binding when HIV-IC* were incubated with serum deprived of Ca^{2+} and Mg^{2+} by adding EDTA. A time-course experiment showed that the adherence of HIV-IC* required exposure to NHS for a minimum of 2 min, when significant binding was already observed; the maximum was reached after 7 min, with no additional changes over the following 30 min (data not shown). These kinetics data confirmed the role of complement in the adherence of HIV-IC* to erythrocytes.

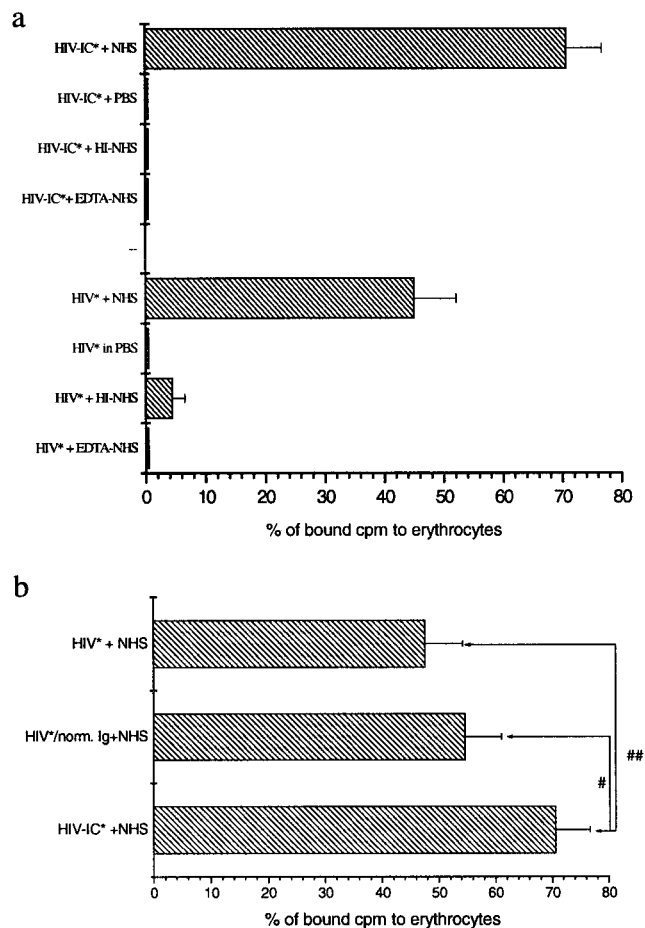


FIGURE 2. a, HIV-IC* and HIV* bind to erythrocytes in the presence of normal human serum. The binding of HIV-IC* and HIV* was abolished in the absence of complement (heat-inactivated serum, EDTA-NHS, or PBS). Error bars indicate the SD of triplicate experiments. b, Comparison of the binding of HIV*, HIV* preincubated with polyclonal IgG, and HIV-IC*; the binding of HIV* alone, or HIV* preincubated with normal polyclonal IgG was significantly lower than that of HIV-IC* (##, $p < 0.01$; #, $p < 0.05$, respectively).

Binding of HIV* to erythrocytes

As a control, SDGU-purified virion particles not exposed to specific anti-HIV Abs were opsonized with NHS and tested for adherence to erythrocytes. Unexpectedly, such complement-exposed virions bound, although with less efficiency compared with HIV-IC* (Fig. 2a). This adherence required complement as well, because it was suppressed in complement-depleted (heat-inactivated) or blocked (EDTA-chelated) sera. The maximal adherence required only a short exposure to serum (7 min). To confirm that the higher binding of HIV-IC* was due to the formation of specific IC, we preincubated HIV* with normal polyclonal IgG devoid of anti-HIV Abs before exposing them to NHS (Fig. 2b). HIV* exposed to normal IgG bound significantly less than HIV-IC* (Fig. 2b). In summary, HIV* alone opsonized in NHS bound significantly to erythrocytes, and this reaction was enhanced in the presence of specific Abs.

To investigate whether HIV* or HIV-IC* bind to thrombocytes in our model system, we performed a control experiment in which we incubated HIV* or HIV-IC* with human platelet-rich plasma under the same conditions used for the erythrocyte adherence assays. We found no significant binding ($\leq 4\%$; data not shown) of NHS-opsonized HIV* or HIV-IC* to platelets. This observation rules out significant binding of HIV* or HIV-IC* to platelets remaining in the erythrocyte preparations.

Complement pathways involved in immune adherence

The next experiments were performed to better define which of the complement pathways was required in the adherence reaction, and whether HIV* and HIV-IC* bind through similar molecular mechanisms.

In the absence of classical pathway function, the adherence of HIV* and HIV-IC* was abolished or reduced (Fig. 3). The absence of HIV-IC* adherence in C1q-deficient serum could be restored to normal when the serum was supplemented with C1q at physiological concentrations. In C2-deficient serum, the adherence was also reduced and could be normalized with C2 for HIV-IC* and improved for HIV*. The incomplete restoration of binding of HIV* in C2-deficient serum repleted with C2 remained unexplained, because this serum had an apparent normal alternative pathway function, as assessed by alternative pathway hemolytic activity assay. Finally, in Mg-EGTA serum, the adherence of HIV-IC* was lower than in NHS. The absence of adherence in C1q-deficient serum was not due to abnormal alternative pathway function, as assessed by hemolytic assay. Moreover, this serum had a normal mannose-binding lectin (MBL) concentration and MBL/mannan-binding lectin-associated serine protease 2 activity (measured by J. Jensenius, University of Aarhus, Aarhus, Denmark); thus, the differences could only be explained by the presence of C1q.

When alternative pathway function was blocked using specific mAbs against factor D, the binding of HIV* was reduced, whereas that of HIV-IC* was little affected. This difference suggests that the alternative pathway amplification loop is necessary for maximal opsonization of HIV*, but not of HIV-IC*.

Binding of HIV* to erythrocytes in hypogammaglobulinemic serum

To further investigate the roles of Igs and natural Abs in the binding of HIV* to erythrocytes, we performed experiments using serum from patients with hypogammaglobulinemia. As a control, the binding of preformed HIV-IC* to erythrocytes behaved as expected in the two hypogammaglobulinemic sera (i.e., binding and abrogation of binding by heat inactivation; data not shown), although the binding was reduced by one-third to one-half compared

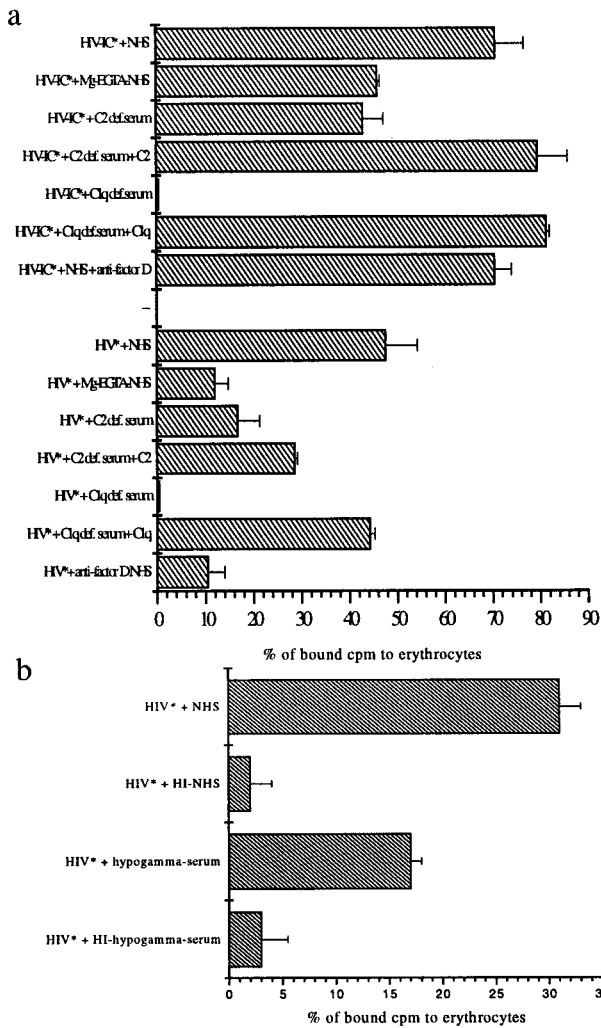


FIGURE 3. *a*, Binding of HIV-IC* and HIV* to erythrocytes in the presence of different sera. The binding for HIV-IC* and HIV* was significantly ($p < 0.05$) lower in C2-deficient serum and Mg-EGTA serum than in NHS. The absence of C1q produced a complete inhibition of binding for HIV-IC* and HIV*. Complement activity in the two deficient sera was restored for HIV-IC* and HIV* by adding the missing component ($p < 0.05$). Human anti-factor D had almost no effect on the binding of HIV-IC*, whereas it induced a significant ($p < 0.05$) loss in the binding of HIV* compared with NHS. *b*, Immune adherence of HIV* in hypogammaglobulinemic serum (in duplicate). This serum induced specific adherence, although less than NHS. The adherence was abolished in heat-inactivated serum. The second hypogammaglobulinemic serum behaved similarly (not shown).

with NHS, probably due to the low complement activity of these sera (50% compared with NHS; see *Materials and Methods*). Of particular interest was that the binding of HIV* alone to erythrocytes was maintained in hypogammaglobulinemic serum and abrogated when the serum underwent heat inactivation, although the binding was again about half normal (Fig. 3*b*). These results suggested that natural Abs were not a requirement for binding of HIV to erythrocytes, i.e., the observed binding was not due to HIV natural Ab ICs, but to the direct interaction of HIV with complement.

Role of CR1 in the adherence of HIV and HIV-IC* to erythrocytes*

To confirm that the adherence of opsonized HIV* and HIV-IC* to erythrocytes occurred via CR1, we incubated them with erythro-

cytes that were preincubated with an excess of anti-CR1 mAbs known to block the C3b/C4b binding sites of CR1. HIV-IC* did not bind to erythrocytes (using Ab 3D9) or bound very weakly (using Ab J3D3; Fig. 4). Interestingly, when added subsequent to the adherence reaction, mAb 3D9 was also capable of inducing the release of a large fraction of HIV-IC* from erythrocytes (>50%; not shown). Finally, no adherence could be observed with sheep erythrocytes, which have no equivalent of CR1.

Role of C1q in adherence of HIV and HIV-IC* to erythrocytes*

Because of the data obtained using C1q-deficient serum, and because C1q is known to bind to HIV directly and has been reported to mediate immune adherence to CR1 (27, 28), we tested whether purified C1q alone would produce adherence in our model. HIV* or HIV-IC* were exposed to increasing concentrations of C1q, then incubated with erythrocytes (Fig. 5). Using different concentrations of C1q, we observed a dose-dependent increase in the adherence of HIV* and HIV-IC*. A significant adherence was seen only at supraphysiological concentrations.

Discussion

The main finding was that free HIV was capable of binding to human erythrocytes in the presence of complement. Indeed, almost 50% of the free virus became immune adherent in our in vitro model. The adherence of HIV-IC was not different from that of many other IC, such as tetanus toxoid/anti-tetanus toxoid and hepatitis B/anti-hepatitis B-IC (25, 29).

Many authors have analyzed the role of complement in HIV infection (5, 7, 30, 31), but to date only a few studies have addressed the adherence of HIV to erythrocytes (6, 32, 33). Lachgar et al. (33) suggested that the Duffy Ag on the membrane of erythrocytes, which is a chemokine receptor, may serve as a binding site for HIV. However, in the absence of serum as a source of complement, we did not observe any direct binding of HIV to erythrocytes, except in the presence of purified C1q, as discussed below. Montefiori et al. (32) have studied the adherence of HIV to CR1 on K562/huCR1-transfected cells and on erythrocytes in the presence of complement and specific Abs. They used p24 Ag as a surrogate

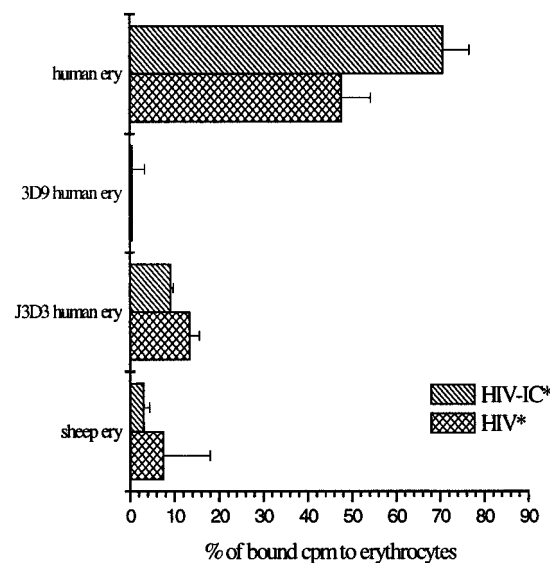


FIGURE 4. Opsonized HIV-IC* and HIV* do not bind to erythrocytes treated with anti-CR1 Abs or sheep erythrocytes. 3D9 and J3D3 anti-CR1 mAbs blocking the C3b/C4b binding site of CR1 inhibited the binding of HIV-IC* or HIV* to human erythrocytes ($p < 0.05$), and there was no significant binding of HIV* or HIV-IC* to sheep erythrocytes ($p < 0.05$).

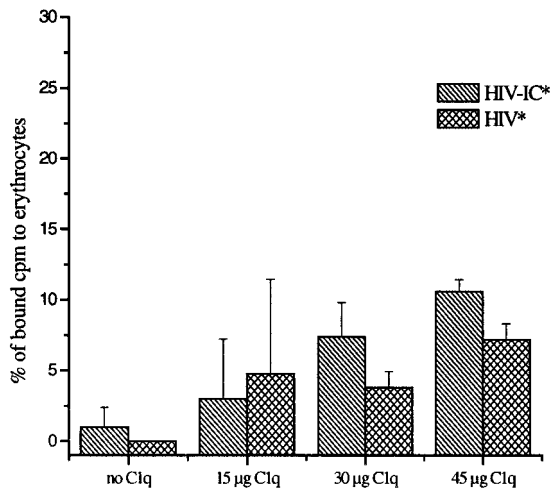


FIGURE 5. Nonopsonized HIV-IC* and HIV* bind to erythrocytes in the presence of purified human C1q. HIV* and HIV-IC* were incubated with purified C1q, starting with a physiological concentration (15 µg) and up to 45 µg. C1q induced the binding of HIV-IC* and HIV* in a dose-dependent manner, which became statistically significant at a 3-fold physiological concentration of C1q ($p < 0.05$).

marker for the presence of virus. Under their experimental conditions some HIV p24 Ag bound to erythrocytes, although the maximum binding was barely $>1\%$ of the total p24 Ag offered. No binding was observed in the absence of complement, CR1, or specific Abs. These experiments suggested that erythrocytes have the property to bind opsonized HIV-IC, not HIV, but with very low efficiency. The differences from our data are probably best explained by the model used. We analyzed only the SDGU fractions known to contain intact viral particles, leaving out soluble proteins and degraded or fragmented viruses. This procedure allowed us to obtain clear evidence of HIV-IC adherence to erythrocytes (up to 80% binding), and we could demonstrate that up to 50% HIV opsonized with normal serum became adherent to erythrocytes as well.

The adherence of HIV and HIV-IC corresponded to the classical immune adherence reaction, because it involved CR1 on erythrocytes. The adherence was blocked in the presence of mAbs known to inhibit the C4/C3 binding sites of CR1. In addition, viruses and HIV-IC prebound to erythrocytes were released upon addition of the same Abs.

The crucial role of the classical pathway in the adherence of HIV as well as HIV-IC was demonstrated by a total lack of adherence in C1q-deficient serum. Using purified complement proteins, Tacnet-Delorme et al. (34) have shown that HIV-1 activates the classical pathway even in the absence of Abs. In our model, the amplification of complement activation by the alternative pathway was also required for an optimal adherence of free HIV, because HIV adherence was very low in factor D-inhibited serum. This contrasted with the adherence of HIV-IC, for which such amplification was not necessary. This should not be surprising, because HIV might have only a limited capacity to activate the classical pathway as opposed to specific Abs fixed to the virus. Interestingly, under conditions that allowed C1q binding to HIV or HIV-IC, but no classical pathway activation (Mg-EGTA and C2-deficient serum), the alternative pathway appeared to have been capable of fixing a sufficient amount of C3 fragments so as to allow some adherence to occur. A possible C2 by-pass system has been described on other occasions (26, 35). An alternative would be that C1q binding to the virus favors alternative pathway activation or

acts as an additional opsonin for binding to erythrocytes. Indeed, in separate experiments we could show that purified C1q by itself mediated some attachment of HIV and HIV-IC to erythrocytes. This finding is in accordance with the results reported by Ebenbichler et al. (27), who demonstrated that C1q binds directly to HIV-1 through specific sites in the transmembrane gp41. C1q is also known to be a ligand for the long homologous repeat-D domain of CR1 (36) and has been shown to be responsible for adherence of IC to erythrocytes in other in vitro models (37). In our study, however, this effect was seen only under high nonphysiological concentrations of C1q.

MBL has been reported to bind HIV and interact with CR1. However, our data do not provide any supporting evidence for an important role of MBL in the interaction between HIV and erythrocytes, because there was no binding of HIV or HIV-IC incubated in C1q-deficient serum, which was determined to have a normal MBL concentration and MBL/MASP-2 activity (measurements performed by Dr. J. Jensenius). Natural Abs have been described in normal sera recognizing the HIV gp120 and protein Tat, however, with low affinity (38, 39). Such naturally occurring Abs might be responsible for activating the classical pathway leading to C3 deposition as well. However, binding of HIV to erythrocytes was maintained in two hypogammaglobulinemic sera, suggesting strongly that such natural Abs do not play a crucial role in the adherence reaction.

When budding out of cells, HIV pinches off some of the cell membrane. Several authors have reported the presence of MCP, decay-accelerating factor, and CD59 (40–42) on virus particles released by human lymphocytes. The infected cells producing HIV virions may thus determine the level of complement activation by the virion, depending on how many of the known complement inhibitors are expressed on the cells. These complement regulatory molecules are expressed at different concentrations on different cells, and their expression may change during cell culture. The presence of complement inhibitors on the viral surface might be modified during the process of budding from the host cells as well. Thus, depending on the cells producing HIV, the virus is more or less capable of activating complement. This might explain some of the reported discrepancies in studies measuring complement activation by HIV virions (34, 43–46). The data we have obtained indicate that the viruses produced by the Hut/4–3 cell line derived from human lymphocytes are complement activating even in the absence of specific Abs.

In conclusion, in our experimental model, both HIV and HIV-IC were capable of activating complement, fixing C3 fragments, and binding to erythrocytes. There is clear evidence of ongoing complement activation in HIV-infected individuals, and the number of CR1 on erythrocytes decreases during the progression of the disease as if CR1 had been consumed, a situation similar to systemic lupus erythematosus and factor I deficiency (6, 47, 48). Our observations suggest that HIV may attach to erythrocytes via complement and CR1 in vivo as well. Because virions attached to cells have been shown to be more stable and infectious than a similar amount of cell-free virus (4, 5), it might be of interest to interfere with this adherence. Finally, the observation that even in the absence of specific Abs the virus can be transported by erythrocytes in the circulation may be of crucial importance at the time of the primary infection.

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