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CD4-independent binding of HIV-1 to the B lymphocyte receptor CR2 (CD21) in the presence of complement and antibody

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SUMMARY

Complement and antibody contribute to infection-enhancement and possible expanded cellular tropism of HIV-1 *in vitro* through a process requiring complement receptors. Until now, however, the ability of HIV-1 to bind complement receptors has not been documented or characterized. We investigated whether antibody and complement permitted HIV-1 to bind to the B lymphocyte receptor, CR2 (CD21), in an effort to learn more about infection-enhancement, and also because CR2 can mediate B cell proliferation and antigen localization in lymphoid organs in other systems. HIV-1 incubated with antibody and fresh human serum as a source of complement bound approximately 10-fold greater to cells expressing CR2 than to HIV-1-permissive cells lacking this receptor. A similar effect was observed using cells which expressed CR2 but no CD4. This binding was minimal in heat-inactivated and C3-deficient sera, and was significantly reduced by the anti-CR2 MoAb, OKB7, but not by the anti-CD4 MoAb, OKT4a. Thus, complement and antibody acted in concert to facilitate the binding of HIV-1 to CR2 independently of CD4. CD4-independent binding of HIV-1 to CR2 was not sufficient to produce infection in Raji-3 cells. Titres of antibodies mediating CR2 binding correlated with antibody titres as measured by immunofluorescence ($P < 0.01$) and infection-enhancement ($P < 0.05$) but were discordant with titres of neutralizing antibodies, a result consistent with the utilization of CR2 for enhanced infection of cells. The ability of complement and antibody to facilitate the binding of HIV-1 to CR2 in the absence of CD4 provides new insights into mechanisms of HIV-1-induced immunopathogenesis and infection-enhancement.

Keywords HIV receptors infection-enhancement immunopathogenesis

INTRODUCTION

Infection with HIV-1, the causative agent of AIDS [1–3], leads to various forms of immune dysfunction followed by the depletion of $CD4^+$ T lymphocytes, which are major targets for infection [4]. Significant and persistent antibody responses are elicited in response to infection, including the generation of *in vitro* neutralizing antibodies [5]. Even so, death from opportunistic infections and neoplasms ultimately ensues. Failure of the immune response to control infection is compounded by the fact that HIV-1-specific antibodies themselves might contribute to immunopathogenesis. Possible mechanisms by which this may occur include polyclonal B cell activation, antigenic mimicry, and infection-enhancement [4, 6].

Some of the immunopathological consequences of HIV-1 infection just mentioned could be influenced by complement and its receptors [7–9]. In support of this, HIV-1 resists complement-mediated lysis [10,11] even though it activates both the classical and alternative complement pathways [12–14]. Further, both complement pathways have been reported to be chronically activated in HIV-1-infected individuals [15–17]. One consequence of complement activation by HIV-1 *in vitro* in the presence of HIV-1-specific antibody is infection-enhancement [18–23]. The fact that complement receptors are needed for this type of infection-enhancement to occur [18–21] suggests that HIV-1 immune complexes bind to them. A recent study demonstrated enhanced binding to monocytes in the presence of complement and antibodies but did not address the role of complement receptors [24]. Therefore, until now, antibody-mediated binding of HIV-1 to complement receptors in the presence of complement has not been demonstrated.

The complement receptor cited most frequently as being needed for infection-enhancement is CR2 (CD21) [19,21,22]. CR2 is a 145-kD transmembrane glycoprotein containing a 34-amino acid cytoplasmic domain, a 24-amino acid transmembrane region, and a 954-amino acid extracellular domain forming filamentous structures which protrude from the plasma membrane and bind ligand at their external terminus [9,25]. It is found in abundance on B lymphocytes, where it acts as receptor

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for Epstein–Barr virus (EBV) and for C3d-containing fragments of complement component, C3 [9,25]. Expression of CR2 on B lymphocytes is temporal, being present during B cell maturation but disappearing when cells are in active proliferation and when terminally differentiated into plasma cells [26]. Immunohistochemical examination of frozen sections has also identified an abundance of CR2 on human follicular dendritic cells (FDC) found in germinal centres of lymph nodes and in tonsils [27]. FDC are associated with the C3-dependent retention of antigen in lymphoid follicles, and with the generation of memory B cells [28]. In addition, CR2 has been found on 30–40% of normal peripheral blood T lymphocytes but the density was at least 10-fold lower than what was found on peripheral blood B lymphocytes [29].

Here, CR2 was shown to be a receptor for HIV-1 in the presence of antibody and complement, and that binding to CR2 did not require the presence of CD4. The possible relationship of these findings to HIV-1-induced immunopathogenesis and infection-enhancement is discussed.

MATERIALS AND METHODS

Cells and virus

The human T lymphoblastoid cell lines MT-2 ($CD4^+$, $CR2^+$), CEM ($CD4^+$, $CR2^-$) and H9, and the HIV-1 strain IIIB [30,31], were obtained from R.C. Gallo (National Cancer Institute, Bethesda, MD). The B lymphoblastoid cell line, Raji-3 ($CD4^-$, $CR2^+$), was provided by Dharam Ablashi (National Cancer Institute). Cells were cultured in RPMI 1640 containing 12% heat-inactivated fetal bovine serum (FBS) and 50 μg gentamycin/ml. Phenotypic expression of CD4 and CR2 on MT-2, Raji-3 and CEM cells was confirmed by live-cell immunofluorescence staining and flow cytometry using saturating amounts of OKT4a-FITC and OKB7-PE (Ortho Diagnostics Systems), respectively.

Sera and MoAbs

Human serum used as a source of complement was obtained as a lyophylized powder from Sigma Chemical Co. (St Louis, MO). Human serum deficient in complement component C3 was obtained from Cappell (West Chester, PA). Both sera were $HIV\text{-}1^-$ by Western blot (DuPont). Heat inactivation was performed at 56°C for 1 h. Preservative-free, unconjugated, OKT4a (anti-CD4) and OKB7 (anti-CR2) MoAbs were obtained from Ortho-Diagnostics Systems (Raritan, NJ). Preparations of MoAbs used here were previously shown to block complement-independent (OKT4a) and complement-mediated (OKB7) HIV-1 and SIVmac251 infection of MT-2 cells in this laboratory [19, 32]. $HIV\text{-}1^+$ sera were from infected or vaccinated individuals and were confirmed positive by ELISA and Western blot. Sera from vaccinated individuals were obtained 2 weeks after rgp160 (VaxSyn, MicroGeneSys Inc, Meriden, CT) boosting of volunteers previously immunized with a recombinant vaccinia virus (HIVAC-1e, Oncogen/Bristol-Myers Squibb, Seattle, WA) expressing gp160 [33]. The vaccinated volunteers were enrolled at Vanderbilt University as part of the NIH-AIDS Vaccine Clinical Trials Network. All sera were heat-inactivated before use.

Preparation and titration of virus

Viral stocks were prepared by washing chronically infected H9/HIV-1 IIIB cells with growth medium and incubating in fresh growth medium for 2 days. Virus-containing culture fluids were made cell-free by low-speed centrifugation and 0·45 μm filtration, and stored in aliquots at −70°C. No attempts were made to futher concentrate virus particles since procedures such as centrifugation or density banding could distort the surface glycoprotein content. Viral concentrations were measured using a commercially available enzyme immunoassay which quantifies HIV-1 p24 core protein (Abbott Diagnostics, Chicago, IL). Infectious titres were defined as 50% tissue culture infectious doses (TCID50) as determined in a microdilution-plate infection assay using MT-2 cells as previously described [34].

HIV-1 binding assay

Viral suspensions (0·5 ml) were incubated for 30 min at 37°C with 0·25 ml of undiluted complement serum (i.e. fresh human serum) or various complement-deficient human sera (1:3 final dilution on virus), with or without diluted $HIV\text{-}1^+$ serum. Portions of these viral suspensions (0·25 ml) were then added to 0·25 ml of cell suspensions in 1·5 ml microcentrifuge tubes in duplicate. Virus particles were allowed to bind to cells for 2 h at room temperature. During this time the cells were kept constantly suspended by having the tubes on a rotating platform. Unadsorbed virus particles were removed at the end of the incubation by washing the cells three times with 5 ml of growth medium. Washed cells were suspended in a solution of 0·5% Triton X-100, cell debris removed by centrifugation, and samples analysed for p24 antigen using the Abbott enzyme immunoassay as described by the manufacturer. Concentrations of p24 were determined from standards included in the kits. All samples of a given experiment were assayed at the same time using reagents from the same kit in order to maintain consistency.

In MoAb blocking experiments, cell suspensions were preincubated for 1 h at room temperature with constant mixing in the presence and absence of MoAbs. Viral suspensions were then added to the cells and incubated as described above.

IFA

Antibody titres were measured by immunofluorescence assay (IFA) using slides of fixed H9/HIV-1 IIIB cells as previously described [35]. Briefly, slides were prepared by air drying and fixing in a 1:1 mixture of acetone:methanol for 30 min. Sera were diluted in PBS containing 0·1% globin-free bovine serum albumin (PBS-BSA) and incubated on slides for 30 min at 36°C and 100% humidity. After two washes in carbonate buffer (0·11 M Na_2CO_3, 0·4 M $NaHCO_3$, 0·15 M NaCl, 0·05% Tween-80), the slides were incubated for 30 min at 36°C and 100% humidity with a 1:200 dilution of fluorescein-conjugated, IgG fraction of goat anti-human IgG (heavy and light chains specific, Cappell) containing Evan's blue counter stain. After two additional washes the slides were mounted with 50% glycerol and examined for fluorescence using a Nikon DIAPHOT-TMD-EF fluorescence microscope. IFA titres were defined as the reciprocal of the last dilution where positive fluorescence was observed.

Neutralizing antibodies

Titres of HIV-1 neutralizing antibodies were measured in 96-well microdilution-plate infection assays using cytopathic effect

(CPE) as infection endpoint as previously described [34]. Briefly, two-fold dilutions of samples were made in triplicate in a total of 100 μl growth medium. Fifty microlitres of virus (5×10^4 $TCID_{50}$/50 μl) were added to all wells except for one row of eight non-cytopathic control wells, which received growth medium in place of virus. MT-2 cells (5×10^4 in 100 μl of growth medium) were then added to each well. Viral-induced CPE was quantified by vital dye (neutral red) uptake in remaining viable cells 3 days later. Per cent protection is defined as the difference in light absorption between test wells (cells + test serum + virus) and virus control wells (cells + virus) divided by the difference in light absorption between cell control wells (cells only) and virus control wells. Plates were harvested when >80% CPE was present in the virus control wells. Neutralizing titres were defined by the reciprocal of the last dilution resulting in 50% or greater protection.

Infection-enhancing antibodies

Titres of HIV-1 infection-enhancing antibodies were measured in MT-2 cells by a modification of the above neutralizing antibody assay [18–20]. Serial dilutions of sera were made in triplicate in 96-well microdilution plates. Diluent was growth medium containing fresh HIV$^-$ human serum diluted 1:20 as a source of complement. Virus (5×10^4 $TCID_{50}$/50 μl per well) was added and incubated for 1 h at 37°C (final dilution of complement on virus was 1:40). MT-2 cells (5×10^4 in 100 μl of growth medium) were then added to each well and the plates incubated until syncytium formation and CPE were observed microscopically. Viable cells were then quantified by neutral red uptake. The range for quantifying per cent viable cells was determined by the difference in light absorption at 540 nm between the average of eight non-enhancing control wells (cells + virus + complement but no enhancing serum) and the average of eight blank wells containing no cells or virus. Plates were harvested when 2–10 syncytia were present in the non-enhancing control wells. Enhancing titres were defined by the reciprocal of the last dilution where >20% CPE was present.

Challenge of Raji-3 cells with HIV-1

In one set of duplicate cultures, virus-containing culture fluid (3×10^6 $TCID_{50}$ in 5 ml) was incubated at 37°C for 30 min with 1:20-diluted fresh human serum as a source of complement, and 1:500-diluted, HIV-1-specific serum no. 2. In another set of duplicate cultures, virus was not incubated with complement or HIV-1-specific serum. Raji-3 cells (2×10^6 in 5 ml of growth medium) were then added to each culture. The cells were washed after overnight incubation and placed in fresh growth medium. They were maintained for 8 weeks, with p24 assays being performed after 1, 3, 6, and 8 weeks.

RESULTS

Concentration-dependent binding of virus to Raji-3 and MT-2 cells

In preliminary studies (data not shown) we determined that HIV-1$^+$ serum promoted the binding of HIV-1 to Raji-3 and MT-2 cells in the presence of 1:3-diluted, fresh HIV$^-$ serum. This led us to suspect that antibody and complement were responsible for the binding. In order to evaluate this phenomenon properly, we first established the limits of detection and the linear range of binding. This was done by adding virus in amounts of 50–31 250 pg p24 to an equal number of Raji-3 or MT-2 cells for each concentration of virus. The amount of virus which bound at each concentration is shown in Fig. 1. Binding was detected even at the lowest input of virus (50 pg p24 added per reaction, where the amount of virus bound was 6·5 and 20 pg p24 for Raji-3 and MT-2 cells, respectively). There was a linear increase in binding to both cell lines as the concentration of input virus increased. Receptor saturation was never reached since the curve remained linear at all concentrations of virus used. All subsequent binding experiments were performed within this linear range.

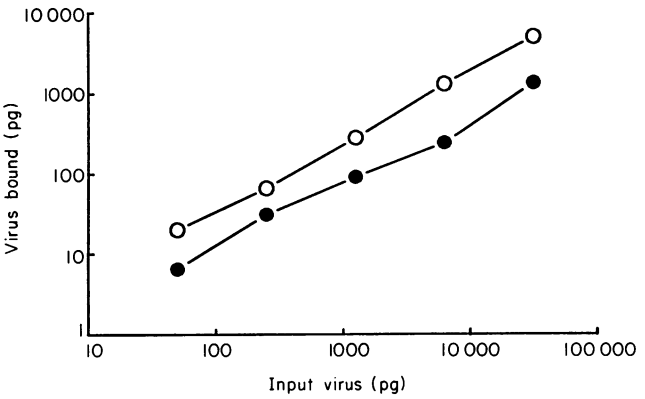

Fig. 1. Virus concentration-dependent binding to Raji-3 (●) and MT-2 cells (○) in the presence of antibody and fresh complement serum. HIV-1 suspensions containing 50–31 250 pg p24 were incubated with a 1:40 dilution of HIV-1$^+$ serum no. 2 and a 1:3 dilution of fresh human complement serum and in a total of 0·25 ml at 37°C for 30 min. These were then added to 0·5 ml of Raji-3 or MT-2 cells (8×10^6) and incubated for binding as described in Materials and Methods. Each point represents an average of two binding reactions.

Binding to CR2 in the presence of complement and antibody

Three different HIV-1$^+$ sera were examined for an ability to promote complement-mediated binding of HIV-1 to Raji-3 cells, which are CD4$^-$, CR2$^+$. Results are shown in Fig. 2. Fresh complement serum alone permitted readily detectable amounts of HIV-1 to bind (16 pg p24). The addition of antibody (1:40 dilution) increased this binding an additional 2·6–3·4 times (closed bars). When C3-deficient serum was used in place of fresh complement serum, the amount of binding was similar to that in the presence of complement alone (open bars). This binding was probably due to antibody in the HIV-1$^+$ serum, together with a small amount of C3 present in the C3-deficient serum.

The dramatic reduction of binding seen in C3-deficient serum indicated that complement was involved, and suggested that complement receptors might be utilized. Also, because Raji-3 and MT-2 cells both express a high density of CR2, and since MT-2 cells do not express two other well characterized complement receptors, CR1 or CR3 [20], CR2 seemed the most likely complement receptor to be involved. Therefore, CR2-specificity of binding was initially examined in experiments using cells which expressed different receptors. Here, antibody promoted the complement-mediated binding of HIV-1 to Raji-3 and MT-2 cells but failed to do so using CEM cells, which are CR2$^-$ (Table 1). The fact that Raji-3 cells are CD4$^-$ indicated that antibody-dependent, complement-mediated binding was independent of CD4. Receptor requirements were delineated

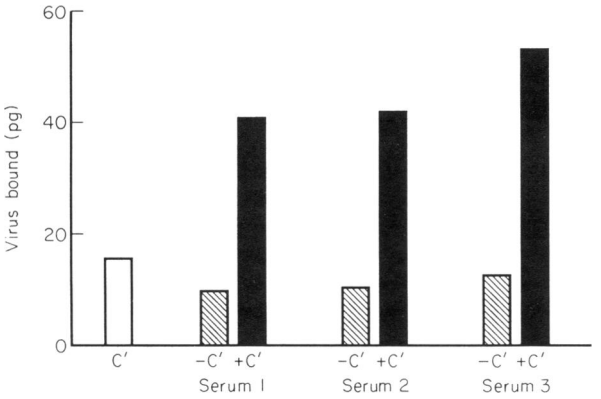

Fig. 2. Antibody-dependent binding of HIV-1 to Raji-3 cells by three different HIV-1$^+$ sera in the presence and absence of complement. HIV-1 IIIB (6250 pg p24) was incubated with a 1:40 dilution of HIV-1$^+$ sera and a 1:3 dilution of either fresh complement serum (+C′) or C3-deficient serum (−C′) in a total of 0·25 ml at 37°C for 30 min. These were then added to 0·5 ml of Raji-3 cells (5×10^6) and incubated for binding as described in Materials and Methods. As a control, one set of binding reactions was done in the presence of complement serum without the addition of HIV-1$^+$ serum (C′). Each point represents an average of two binding reactions.

further using MoAbs to CD4 (OKT4a) and CR2 (OKB7) in blocking experiments. A much greater dilution of HIV-1$^+$ serum was used in these experiments in order to enhance the sensitivity of blocking. Here, OKB7 reduced the amount of binding to Raji-3 and MT-2 cells in the presence of antibody and complement by 80% and 100%, respectively, relative to the amount of binding which occurred in the presence of complement alone (Table 1). In contrast, the same concentration of OKT4a failed to block binding at all. These results confirm the binding of HIV-1 to CR2, independently of CD4.

Comparison of antibody titres

A method for quantifying the titre of antibodies which promoted complement-mediated binding of HIV-1 to CR2 was developed. Here, five-fold dilutions of HIV-1$^+$ sera were used for binding measurements. An example of a binding curve is shown for serum no. 1 in Fig. 3. The amount of binding in the presence of complement alone is represented by a dashed line. Initially it was thought that the amount of binding at one serum dilution might be used to represent the titre. Interestingly, however, a saturating amount of antibody was possible, where equal binding occurred at the two lowest dilutions for serum no. 2. To avoid having values fall within this range, we decided to define the titre as the reciprocal of the last dilution where p24 was detected, multiplied by the amount of p24 (pg) present at that dilution (after subtracting the amount of complement-mediated binding in the absence of antibody). Titres for sera from six different HIV-1-infected individuals, and from two uninfected, gp160-immunized individuals are shown in Table 2. Representative sera were chosen which had low to high antibody titres by IFA, neutralization and enhancement. Titres showed a statistically significant correlation with antibody titres as measured by IFA and enhancement ($P < 0{\cdot}01$ and $P < 0{\cdot}05$, respectively) but had no correlation with neutralizing antibody titres ($P > 0{\cdot}1$).

Effect of complement and antibody on infectibility of Raji-3 cells

Raji-3 cells were unable to be infected with HIV-1 IIIB whether or not the virus had first been incubated with complement and a 1:500 dilution of enhancing serum. Infections were monitored by p24 immunoassay for 8 weeks. The enhancing serum used in this experiment (serum no. 2) had a neutralizing titre of 256 and an enhancing titre of 17496 in MT-2 cells (Table 2). The neutralizing titre of this serum was reduced to 64 in the presence of complement, where at a 1:500 dilution it produced dramatic infection-enhancement in MT-2 cells within 3 days (data not shown).

DISCUSSION

CR2 was shown to be a CD4-independent receptor for HIV-1 in the presence of complement and HIV-1-specific antibody. This was demonstrated in a biological binding assay using live cells and a preparation containing whole infectious virus particles, where virus binding was quantified by p24 immunoassay. CR2 was identified as a receptor by showing that, in the presence of complement and antibody, HIV-1 bound to cells expressing CR2 but no other complement receptors and, in the case of Raji-3 cells, no CD4. Further, binding to CR2$^+$ cells was partially blocked by OKB7. The inability of saturating amounts of OKB7 to block all complement-mediated binding (Table 1) probably reflects minor structural differences in the region of CR2 recognized by OKB7 and natural ligands for CR2 [36].

Complement and antibody increased the amount of virus which bound to CR2$^+$ cells by at least 10-fold over background. In addition, the amount of CR2-mediated binding to MT-2 (CR2$^+$, CD4$^+$) and Raji-3 (CR2$^+$, CD4$^-$) cells was much greater than CD4-mediated binding to CEM (CR2$^-$, CD4$^+$) cells (Table 1). Possible differences in receptor density might have accounted for this. However, flow cytometric analysis using saturation amounts of anti-CD4 and anti-CR2 MoAbs indicated that there was more CD4 on CEM cells than CR2 on Raji-3 cells (data not shown). Another possible explanation is that HIV-1 particles were lysed by complement, releasing p24 into the medium, and allowing p24 immune complexes to bind to the cells. This was disproved by showing that envelope-specific antibodies in sera from gp160-vaccinated individuals, which contained no anti-p24 antibodies, were reactive in the binding assay (Table 1).

The ability of HIV-1 to bind CR2 in the presence of complement and HIV-1-specific antibody, together with the correlation between titres of binding antibodies and titres of complement-mediated, infection-enhancing antibodies (Table 2), suggests that this is an important feature of *in vitro* infection-enhancement. For example, CR2 might facilitate the entry of virus particles into cells. Results of infection experiments indicate that CD4 would be required together with CR2 in order for entry to occur [19,21,22]. In contrast, Boyer *et al.* provided evidence that MT-2 cells could be infected through CR2, independently of CD4 [13]. The ability of HIV-1 to bind CR2 in the absence of CD4 (Table 1) would seem to support their results. However, we attempted to infect Raji-3 cells at a high multiplicity of infection (> 1) in the presence of complement and antibody and failed to detect infection by p24 immunoassay for up to 8 weeks. The HIV-1-specific serum chosen for this experiment, and the dilution of the serum used, produced strong

Table 1. CR2-specificity of complement-mediated, antibody-dependent binding of HIV-1 to CR2$^+$ cells

Preincubations*	HIV-1 bound (pg p24)†		
	Raji-3 (CD4$^-$/CR2$^+$)	MT-2 (CD4$^+$/CR2$^+$)	CEM (CD4$^+$/CR2$^-$)
Heat-inactivated complement	4	7	<3
Fresh complement	14	54	5
Fresh complement + HIV$^+$ serum	43	109	5
Fresh complement + HIV$^+$ serum + OKT4a	48	111	NT‡
Fresh complement + HIV$^+$ serum + OKB7	20	39	NT

* HIV-1 suspensions (2500 pg p24 for the Raji-3 experiment and 5625 pg p24 for the MT-2 and CEM experiments) were incubated with various complement sera with or without HIV$^+$ serum no. 2 (1 : 1000 dilution) for 30 min before the addition of cells (4×10^6 Raji-3 cells, 1×10^6 MT-2 cells, $3{\cdot}8 \times 10^6$ CEM cells). Cells were incubated in the presence and absence of OKT4a (56 μg/ml) or OKB7 (56 μg/ml), before addition to virus. Bound virions were quantified by enzyme immunoassay as described in Materials and Methods.

† Each p24 value is the average of two binding reactions.

‡ NT, Not tested.

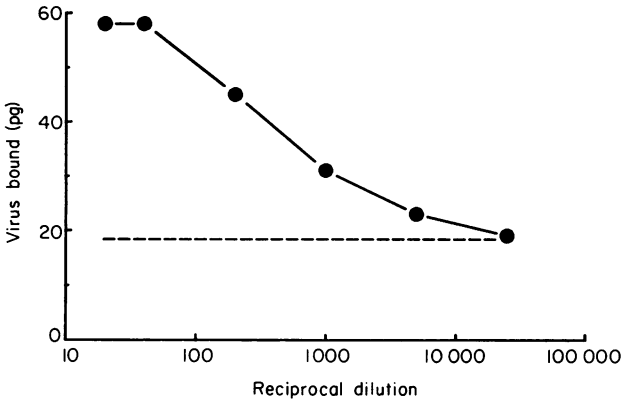

Fig. 3. Titration of antibodies promoting complement-mediated binding of HIV-1 to Raji-3 cells. Virus (1250 pg p24) was incubated with various dilutions of HIV-1$^+$ serum no. 2 and a 1 : 3 dilution of fresh complement serum in 0·25 ml at 37°C for 30 min. Dilutions of serum no. 2 were made in heat-inactivated fetal bovine serum (FBS) before adding to each tube so that the serum protein concentration was always equivalent. As a control, one set of binding reactions was done in the presence of complement serum without the addition of HIV-1$^+$ serum (- - - -). After incubation the entire contents were added to 0·5 ml of Raji-3 cells (8×10^6) and incubated for binding as described in Materials and Methods. Each point represents an average of two binding reactions.

Table 2. Comparison of HIV-1 antibody titres determined by CR2 binding, IFA, neutralization, and infection-enhancement

Serum*	CR2 binding†	Antibody titre (reciprocal dilution)		
		IFA	Neutralization	Enhancement
1	11 000	9720	32	5832
2	10 000	3240	256	17 496
3	25 000	29 160	128	17 496
4	1400	360	<4	72
5	2500	1080	8	540
6	<30	40	32	180
7	3120	640	128	80
8	1600	160	<16	80

* Sera 1–6 were from HIV-1-infected individuals, while sera 7 and 8 were from gp160-vaccinated individuals. All sera were ELISA positive for HIV-1. Sera from infected individuals were positive for all major HIV-1 antigens by Western blot. Sera from vaccinated individuals had antibodies to gp160, gp120 and gp41 but no other HIV-1 antigens by Western blot.

† CR2 binding titres are defined as the reciprocal of the last dilution where p24 was detected multiplied by the amount of p24 (pg) present at that dilution (after subtracting the amount of complement-mediated HIV-1 binding in the absence of antibody). Binding was performed using Raji-3 cells ($3{\cdot}75 \times 10^6$) and HIV-1 IIIB (1250 pg p24) per duplicate binding reaction.

infection-enhancement in MT-2 cells within 3 days. These results indicate that utilization of CR2 as receptor for HIV-1 in the absence of CD4 will not always be sufficient for infection.

Another role for CR2 in infection-enhancement could be in eliciting a signal transduction mechanism, facilitating replication post entry. For example, ligands which bind to CR2 on B lymphocytes have been shown to stimulate the proliferation of activated B lymphocytes in association with increases in intracellular free Ca^{2+} concentrations [37–39]. In this regard, HIV-1 replication has been shown to be increased by stimuli which mobilize intracellular calcium reserves and activate NFkB [40,41].

A putative signal transduction mechanism linked to CR2 could have other consequences for HIV-1 pathogenesis. For example, polyclonal B cell activation is common in infected individuals [6,42]. It is conceivable that HIV-1 immune complexes could drive the proliferation of activated B cells through their interaction with CR2. Another important consideration is the role that complement and its receptors play in localizing antigens in lymphoid organs [28,42]. For example, FDC express a high density CR1, CR2 and CR3 [27], all of which are receptors for particulate antigens containing activated fragments of C3. Therefore, the antibody-dependent interaction of HIV-1 with complement could contribute to the long-term retention of virus on the surface of FDC. Also, HIV-1 bound to

the surface of CR2+ B lymphocytes could transport virus to lymphoid organs during periods of active viral replication. Such localization of HIV-1 in lymphoid organs could contribute to virus spread by facilitating the infection of CD4+ T cells migrating to lymphoid organs in response to infection [43–45]. It might also facilitate the infection of some resident FDC which co-express CD4 [46] and CR2 [27]. These hypotheses are consistent with previous results identifying lymphoid organs as major reservoirs for HIV-1 [47–49], and as sites of active HIV-1 replication late in the course of disease [50].

The ability of CR2 to function as a receptor for HIV-1 in the presence of antibody and complement provides new insight into possible mechanisms contributing to HIV-1-induced immunopathogenesis. The fact that both complement pathways have been shown to be chronically activated in infected individuals [15–17] lends support to the potential clinical relevance of these findings. Since the cell lines used here did not express other complement receptors, it remains to be seen whether complement receptors other than CR2 can act as HIV-1 receptors.

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REFERENCES

1 Kalyanaraman VS, Cabradilla CD, Getchell JP *et al.* Antibodies to the core protein of lymphadenopathy virus (LAV) in patients with AIDS. Science 1984; **225**:321–3.
2 Kaminsky LS, McHugh T, Stites D, Volberding P, Henle G, Henle W, Levy JA. High prevalence of antibodies to acquired immune deficiency syndrome (AIDS)-associated retrovirus (ARV) in AIDS and related conditions but not in other disease states. Proc Natl Acad Sci USA 1985; **82**:5535–9.
3 Sarngadharan MG, Popovic M, Bruch L, Schupbach J, Gallo RC. Antibodies reactive with human T-lymphotropic retroviruses (HTLV-III) in the serum of patients with AIDS. Science 1984; **224**:506–8.
4 Rosenberg ZF, Fauci A. The immunopathogenesis of HIV infection. Adv Immunol 1989; **47**:377–431.
5 Robert-Guroff M, Brown M, Gallo RC. HTLV-III neutralizing antibodies in patients with AIDS and AIDS-related complex. Nature 1985; **316**:72–74.
6 Amadori A, Chieco-Bianchi L. B-cell activation and HIV-1 infection: deeds and misdeeds. Immunol Today 1991; **11**:374–9.
7 Muller-Eberhard HJ. Molecular organization and function of the complement system. Ann Rev Biochem 1988; **57**:321–47.
8 Erdei A, Fust G, Gergely J. The role of C3 in the immune response. Immunol Today 1991; **12**:332–7.
9 Ahearn JM, Fearon DT. Structure and funtion of the complement receptors, CR1 (CD35) and CR2 (CD21). Adv Immunol 1989; **46**:183–219.
10 Harada S, Yoshiyama H, Yamamoto N. Effect of heat and fresh human serum on the infectivity of human T-cell lymphotropic virus type III evaluated with new bioassay systems. J Clin Microbiol 1985; **22**:908–11.
11 Banapour B, Sernatinger J, Levy JA. The AIDS-associated retrovirus is not sensitive to lysis or inactivation by human serum. Virol 1986; **152**:268–71.
12 Reisinger EC, Vogetseder W, Berzow D *et al.* Complement-mediated enhancement of HIV-1 infection of the monoblastoid cell line U937. AIDS 1990; **4**:961–5.
13 Boyer V, Desgranges C, Trabaud M-A, Fischer E, Kazatchkine MD. Complement mediates human immunodeficiency virus type 1 infection of a human T cell line in a CD4- and antibody-independent fashion. J Exp Med 1991; **173**:1151–8.
14 Ebenbichler CF, Thielens NM, Vornhagen R, Marschang P, Arlaud GJ, Dierich MP. Human immunodeficiency virus type 1 activates the classical pathway of complement by direct C1 binding through specific sites in the transmembrane glycoprotein gp41. J Exp Med 1991; **174**:1417–24.
15 Tausk FA, McCutchan JA, Spechko P, Schreiber RD, Gigli I. Altered erythrocyte C3b receptor expression, immune complexes, and complement activation in homosexual men in varying risk groups for acquired immune deficiency syndrome. J Clin Invest 1986; **78**:977–82.
16 Perricone R, Fontana L, DeCarolis C, Carini C, Sirianni MC, Aiuti F. Evidence for activation of complement in patients with AIDS related complex (ARC) and/or lymphadenopathy syndrome (LAS). Clin Exp Immunol 1987; **70**:500–7.
17 Senaldi G, Peakman M, McManus T, Davies ET, Tee DEH, Vergani D. Activation of the complement system in human immunodeficiency syndrome infection: relevance of the classical pathway to pathogenesis and disease severity. J Infect Dis 1990; **162**:1227–32.
18 Robinson WE, Montefiori DC, Mitchell WM. Antibody-dependent enhancement of human immunodeficiency virus type 1 infection. Lancet 1988; **i**:790–4.
19 Robinson WE, Montefiori DC, Mitchell WM. Complement-mediated antibody-dependent enhancement of HIV-1 infection requires CD4 and complement receptors. Virology 1990; **175**:600–4.
20 Robinson WE, Montefiori DC, Gillespie DH, Mitchell WM. Complement-mediated, antibody-dependent enhancement of HIV-1 infection *in vitro* is characterized by increased protein and RNA synthesis and infectious virus release. J Acquir Immune Defic Syndr 1989;**2**:33–42.
21 Tremblay M, Meloche S, Sekaly R-P, Wainberg MA. Complement receptor type 2 mediates enhancement of human immunodeficiency virus infection in Epstein–Barr virus-carrying B cells. J Exp Med 1990; **171**:1791–6.
22 Gras GS, Dormont D. Antibody-dependent and antibody-independent complement-mediated enhancement of human immunodeficiency virus type 1 infection in a human, Epstein–Barr virus-transformed B-lymphocytic cell line. J Virol 1991; **65**:541–5.
23 June RA, Schade SZ, Bankowski MJ *et al.* Complement and antibody mediate enhancement of HIV infection by increasing virus binding and provirus formation. AIDS 1991; **5**:269–74.
24 Bakker LJ, Nottet HSLM, de Vos M, de Graaf L, Van Strijp JAG, Visser MR, Verhoef J. Antibodies and complement enhance binding and uptake of HIV-1 by human monocytes. AIDS 1992; **6**:35–41.
25 Cooper NR, Moore MD, Nemerow GR. Immunobiology of CR2, the B lymphocyte receptor for Epstein–Barr virus and the C3d complement fragment. Ann Rev Immunol 1988; **6**:85–113.
26 Tedder TF, Clement LY, Cooper MD. Expression of C3d receptors during human B cell differentiation: immunofluorescence analysis with the HB-5 monoclonal antibody. J Immunol 1984; **133**:678–83.
27 Reynes M, Aubert JP, Cohen JHM, Audouin J, Tricottet V, Diebold J, Kazatchkine MD. Human follicular dendritic cells express CR1, CR2, and CR3 complement receptor antigens. J Immunol 1985; **135**:2687–94.
28 Klaus GGB, Humphrey JH, Kunkl A, Dongworth DW. The follicular dendritic cell: its role in antigen presentation and in the generation of immunological memory. Immunol Rev 1980; **53**:3–28.
29 Fischer E, Delibrias C, Kazatchkine MD. Expression of CR2 (the

C3dg/EBV receptor, CD21) on normal human peripheral blood T lymphocytes. Immunol 1991; **146**:865–9.

30 Popovic M, Sarngadharan MG, Read E, Gallo RC. Detection, isolation and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. Science 1984; **224**:497–500.

31 Wain-Hobson S, Vartanian J-P, Henry M *et al.* LAV revisited: origins of the early HIV-1 isolates from Institut Pasteur. Science 1991; **252**:961–5.

32 Montefiori DC, Robinson WE, Hirsch VM, Modliszewski A, Mitchell WM, Johnson PR. Antibody-dependent enhancement of simian immunodeficiency virus infection *in vitro* by plasma from SIV-infected rhesus macaques. J Virol 1990; **64**:113–9.

33 Graham BS, Matthews TJ, Belshe RB *et al.* Augmentation of HIV-1 neutralizing antibody by priming with gp160 recombinant vaccinia and boosting with rgp160. J Infect Dis. In press.

34 Montefiori DC, Robinson WE, Schuffman SS, Mitchell WM. Evaluation of antiviral drugs and neutralizing antibodies against human immunodeficiency virus by a rapid and sensitive microtiter infection assay. J Clin Microbiol 1988; **26**:231–5.

35 Montefiori DC, Mitchell WM. Infection of the HTLV-II-bearing T cell line C3 with HTLV-III/LAV is highly permissive and lytic. Virology 1986; **155**:726–31.

36 Martin DR, Yuryev A, Kalli KR, Fearon DT, Ahearn JM. Determination of the structural basis for selective binding of Epstein–Barr virus to human complement receptor type 2. J Exp Med 1991; **174**:1299–311.

37 Matsumoto AK, Kopicky-Burd J, Carter RH, Tuveson DA, Tedder TF, Fearon DT. Intersection of the complement and immune systems: a signal transduction complex of the B lymphocyte-containing complement receptor type 2 and CD19. J Exp Med 1991; **173**:55–64.

38 Carter RH, Spycher MO, Ng YC, Hoffman R, Fearon DT. Synergistic interaction between complement receptor type 2 and membrane IgM on B lymphocytes. J Immunol 1988; **141**:457–63.

39 Bohnsack JF, Cooper NR. CR2 ligands modulate human B cell activation. J Immunol 1988; **141**:2569–76.

40 Cullen BR, Green WC. Regulatory pathways governing HIV-1 replication. Cell 1989; **58**:423–6.

41 Harbison MA, Kim S, Gillis JM, Hammer SM. Effects of the calcium channel blocker verapamil on human immunodeficiency virus type 1 replication in lymphoid cells. J Infect Dis 1991; **164**:53–60.

42 Martinez-Maza O, Crabb E, Mitsuyasu RT, Fahey JL, Giorgi JV. Infection with the human immunodeficiency virus (HIV) is associated with an *in vivo* increase in B lymphocyte activation and immaturity. J Immunol 1987; **138**:3720–4.

43 Parrot DMV, Wilkinson PC. Lymphocyte locomotion and migration. Prog Allergy 1981; **28**:193–284.

44 Jacob J, Kelsoe G, Rajewsky K, Weiss U. Intraclonal generation of antibody mutants in germinal centres. Nature 1991; **354**:389–92.

45 Rouse RV, Ledbetter JA, Weissman IL. Mouse lymph node germinal centers contain a selected subset of the T cell-helper phenotype. J Immunol 1982; **128**:2243–6.

46 Knight SC, Patterson S. Effect of human immunodeficiency virus on dendritic cells isolated from human peripheral blood. In: DuPont B, ed. Proceedings of Tenth Histocompatibility Workshop, Berlin: Springer Verlag 1990:378–82.

47 Pantaleo G, Graziosi C, Butini L, Pizzo PA, Schnitman SM, Kotler DP, Fauci AS. Lymphoid organs function as major reservoirs for human immunodeficiency virus. Proc Natl Acad Sci USA 1991; **88**:9838–42.

48 Tenner-Racz K, Racz P, Bofill M *et al.* HTLV-III/LAV viral antigens in lymph nodes of homosexual men with persistent generalized lymphadenopathy and AIDS. Am J Pathol 1986; **123**:9–15.

49 Spiegel H, Herbst H, Niedobitek G, Foss H-D, Stein H. Follicular dendritic cells are a major reservoir for human immunodeficiency virus type 1 in lymphoid tissues facilitating infection of $CD4^+$ T-helper cells. Am J Pathol 1992; **140**:15–22.

50 Fox CH, Tenner-Racz K, Racz P, Firpo A, Pizzo PA, Fauci AS. Lymphoid germinal centers are reservoirs of human immunodeficiency virus type 1 RNA. J Infect Dis 1991; **164**:1051–7.