

# Complement Mediates Human Immunodeficiency Virus Type 1 Infection of a Human T Cell Line in a CD4- and Antibody-independent Fashion

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## Summary

Incubation of the human T cell lymphotropic virus (HTLV)-IIIB and HTLV-RF strains of human immunodeficiency virus type 1 (HIV-1) with normal seronegative human serum under conditions that allow complement activation resulted in enhancement of infection of the MT2 human T cell line cultured in the presence of low amounts of virus. Infection of MT2 cells was assessed by measuring reverse transcriptase activity in supernatants at day 9 of culture. Complement activation by viral suspensions occurred through the alternative pathway. Opsonization of HTLV-RF viral particles with complement was sufficient to allow a productive infection to occur in cells exposed to suboptimal amounts of virus. Infection of MT2 cells with suboptimal amounts of serum-opsonized HIV-1 was suppressed by blocking the C3dg receptor (CR2, CD21) on MT2 cells with monoclonal anti-CR2 antibody and rabbit F(ab')<sub>2</sub> anti-mouse immunoglobulin antibodies. Blocking of the gp120-binding site on CD4 under similar experimental conditions had no inhibitory effect on infection of MT2 cells with opsonized virus. Opsonization of HIV-1 with seronegative serum also resulted in a CR2-mediated enhancement of the infection of normal peripheral blood mononuclear cells and T lymphocytes. These results indicate that complement in the absence of antibody may enhance infection of C3 receptor-bearing T cells with HIV-1, and that the interaction of opsonized virus with the CR2 receptor may result by itself in the infection of target T cells in a CD4- and antibody-independent fashion.

The CD4 molecule functions as a high affinity cellular receptor for the gp120 envelope protein of HIV-1 (1–3). The binding site of gp120 on CD4 has been mapped using mAbs directed against human CD4 and site-directed mutagenesis of the CD4 gene (4). CD4 serves as the primary receptor for HIV on T lymphocytes and may be important for viral entry into CD4<sup>+</sup> monocyte/macrophages (5–8). However, a number of phenotypically and/or genomically CD4<sup>-</sup> human cells may be infected with HIV in vitro (9–18), implicating that alternative pathways for viral entry should be considered.

A role for lymphocyte function-associated antigen type 1 (LFA-1)<sup>1</sup> (CD11a/CD18) in the fusion process of HIV with target cell membranes has recently been suggested (19, 20). There is also evidence for a role of anti-HIV antibodies and Fc receptors on monocyte/macrophages in enhancing viral

binding and infection (21), and for an enhancing role of antibody and complement in the infection of the MT2 T cell line (22) and of EBV-transformed human B cells (23, 24).

Retroviruses activate human complement (25–29). Intact HIV (30), recombinant gp160 of HIV (Thieblemont, N., N. Haeffner-Cavaillon, L. Weiss, F. Maillet, and M. D. Kazatchkine, manuscript submitted for publication), and HIV-infected cells (31) activate complement in whole human serum resulting in cleavage of C3 and deposition of C3 fragments on the viral surface. Surface-fixed fragments of C3 may interact with specific C3 receptors on a variety of cells, including monocyte/macrophages, B cells, and T cells (32). 10% of normal peripheral blood T lymphocytes express the C3b receptor, CR1 (CD35) (33, 34); ~40% of T cells express low amounts of CR2 (C3dg/EBV receptor, CD21) (35).

The present study demonstrates that the infection of the CR2-positive human T cell line MT2 with HIV-1 in the presence of low amounts of virus is greatly enhanced when HIV is pre-opsonized with seronegative human serum. Infection of MT2 cells with serum-opsonized virus occurred in an

<sup>1</sup> Abbreviations used in this paper: HTLV, human T cell lymphotropic virus; IU, infectious units; LFA-1, lymphocyte function-associated antigen type 1; MOI, multiplicity of infection; NHS, normal human serum; RT, reverse transcriptase.

antibody- and CD4-independent fashion. Opsonization of HIV with seronegative serum also enhanced infection of normal human PBMC and T lymphocytes. The enhancing effect of complement was mediated by the interaction of opsonized virus with the CR2 receptor on target T cells.

## Materials and Methods

**Sera and Antibodies.** Normal human serum (NHS) was obtained from three healthy individuals seronegative for HIV-1 and HIV-2. C2-deficient serum was from a homozygous-deficient patient. Factor B-depleted human serum and normal guinea pig serum were purchased from Sigma Chemical Co., St. Louis, MO.

mAb OKT4A of the IgG 2a isotype (Ortho Diagnostic Systems, Raritan, NJ) directed against the HIV binding site-related epitope of human CD4, IgG2a mAb BL4 directed against human CD4 (kindly provided by J. Brochier, Montpellier, France), IgG2a mAb HB5 directed against human CR2 (CD21) (Becton Dickinson & Co., Mountain View, CA), and rabbit F(ab')<sub>2</sub> antibodies against mouse Ig (Cappel Laboratories, Cochranville, PA) were obtained as indicated.

**Target Cells.** The MT2 cell line is highly sensitive to the cytopathic effect of HIV-1 (36). MT2 cells express CD4 and CR2 (CD21) antigens (37). The cell line was maintained in RPMI 1640 (Gibco-BRL, Cergy, France) supplemented with 10% heat-inactivated FCS (Biosys, Compiègne, France) and antibiotics. For experiments using PBMC as targets cells for infection, PBMC were isolated from whole blood of HIV-1-seronegative individuals on Ficoll-Hypaque. Purified T cell lymphotropic (CD2<sup>+</sup> cells) were obtained from PBMC by rosetting with 2-AET-treated sheep erythrocytes. The cells contained >95% CD3<sup>+</sup> cells and <1% CD20<sup>+</sup> cells. Cells were stimulated with PHA (9 µg/ml) (Wellcome Laboratories, Beckenham, UK) for 3 d and then cultured in fresh culture medium containing rIL-2 (20 U/ml) (Boehringer, Mannheim, FRG).

**Sources of Viruses.** The human T cell lymphotropic virus (HTLV)-IIIB and HTLV-RF isolates of HIV-1 were cultivated in H9 cells. Virus was obtained from supernatants of de novo infected cells, clarified from cells by low-speed centrifugation at 400 g for 10 min, passed through a 0.45-µm filter membrane, and stored at -80°C until use. In coculture experiments, the sources of virus were PBMC from two HIV-1-infected patients.

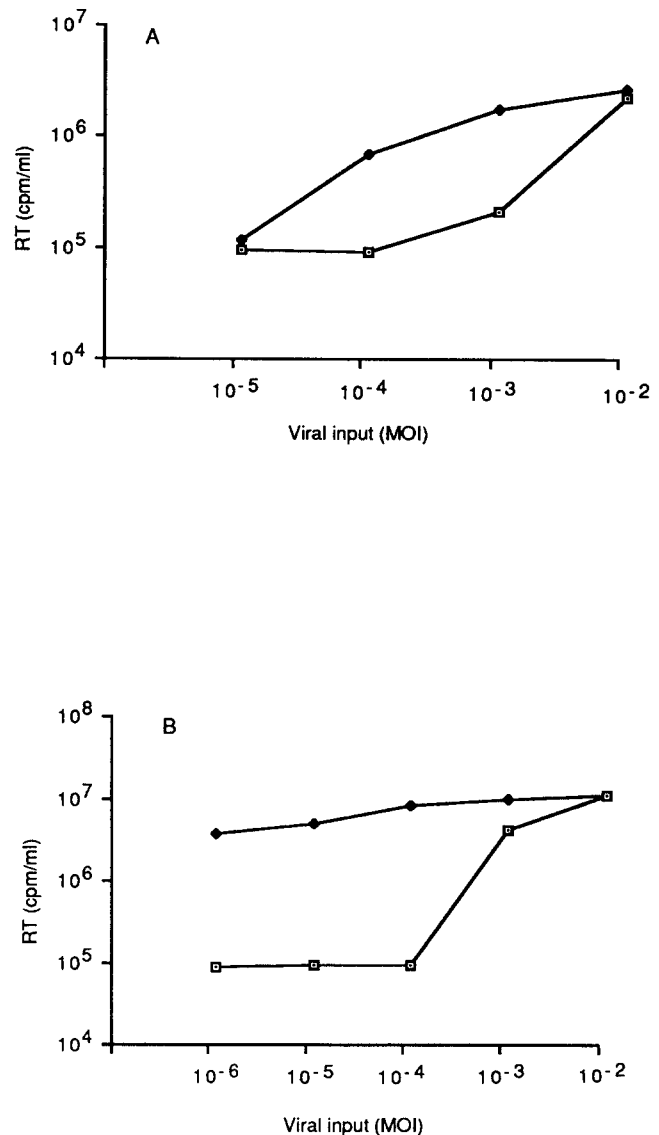
**Viral Titration.** To determine the infectious titer of viral suspensions, H9 cells (10<sup>5</sup> cells in 0.2 ml of culture medium) were infected with 0.1 ml of serial viral dilutions; 300 µl of culture medium was added daily to the cultures as described in reference 38. After 10 d of culture, the highest viral dilution that yielded reverse transcriptase (RT) activity (39) that was at least 20-fold above background activity was defined as the infectious titer of the viral suspension. The titers of HTLV-IIIB and HTLV-RF stock suspensions were 10<sup>5</sup> and 10<sup>6</sup> infectious units (IU)/ml, respectively. Multiplicity of infection (MOI) defined the number of IU per cell used for infection.

**Infection of MT2 Cells with HIV-1.** Infection of MT2 cells was performed in 24-well plates. HIV-1 at appropriate dilutions in culture medium (500 µl final volume) was incubated in the presence or absence of 25 µl of normal human seronegative serum for 1 h at 37°C. MT2 cells (3 × 10<sup>5</sup> cells in 1.5 ml of culture medium) were then added to the mixture and incubated overnight in 5% CO<sub>2</sub> at 37°C. Cells were washed once with Hank's balanced solution (Gibco-BRL), resuspended in 2.0 ml of fresh culture medium, and cultured in another 24-well plate in the presence of 5% CO<sub>2</sub>

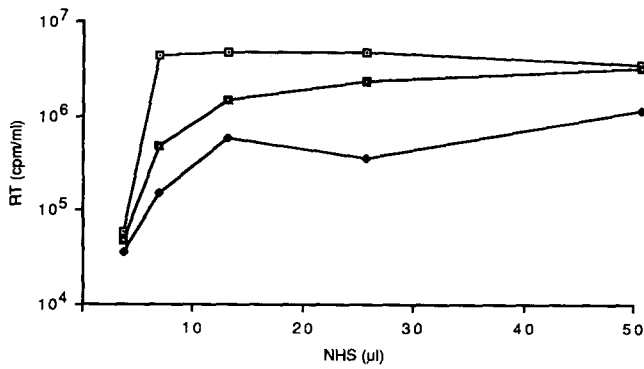
at 37°C. Cultures were examined daily under microscope for the cytopathic effect of HIV. At day 9 of culture, the supernatants were collected and tested for RT activity.

## Results

**Complement-mediated Enhancement of Infection of MT2 Cells with HIV-1.** The enhancing effect of seronegative human serum on infection of MT2 cells with the HTLV-III-RF and HTLV-IIIB strains of HIV-1 is shown in Fig. 1. In the experiments depicted, viral particles were preincubated either with a fixed amount of serum or with culture medium for



**Figure 1.** Enhancement by seronegative human serum of infection of MT2 cells with HIV-1. MT2 cells were infected with increasing amounts of the HTLV-IIIB (A) or HTLV-RF (B) strain of HIV-1 that had been preincubated with seronegative human serum (◆) (25 µl in 500 µl of viral suspension) or with culture medium (□) for 1 h at 37°C. RT activity was assessed on day 9 of culture. MOI equals the number of viral infectious units per cell.



**Figure 2.** Enhancement by seronegative human serum HIV infection of MT2 cells. A fixed concentration of HTLV-III<sub>B</sub> (MOI  $10^{-3}$ ) was preincubated with increasing amounts of serum from three seronegative healthy individuals (NHS) before using the virus for infection of MT2 cells. RT activity was assessed on day 9 of culture.

1 h at 37°C before the virus was used to infect MT2 cells. Infection was assessed by measuring RT activity of the culture supernatants at day 9 of the culture. The enhancing effect of serum was observed at low inputs of virus (MOI  $<10^{-3}$ ); it was not seen at high viral inputs (MOI  $>10^{-2}$ ). Maximal enhancement of HIV infection that was achieved was 10-fold with the HTLV-III<sub>B</sub> strain used at MOI of  $10^{-3}$  and 50-fold with the HTLV-RF strain at MOI of  $10^{-4}$ . Cytopathic changes with the appearance of multinuclear giant cells due to cell fusion were observed as early as day 5 in cultures of cells infected with serum-opsonized virus. The changes occurred at day 7 or later in cultures of cells infected with nonopsonized virus. Fig. 2 shows that the enhancing effect of normal seronegative serum on HIV infection of MT2 cells was dose dependent.

To demonstrate that the serum-mediated enhancing effect that we observed was dependent on complement activation, experiments similar to those depicted in Fig. 1 were performed using EDTA-chelated serum or sera deficient in classical pathway or in alternative pathway proteins. Table 1 shows

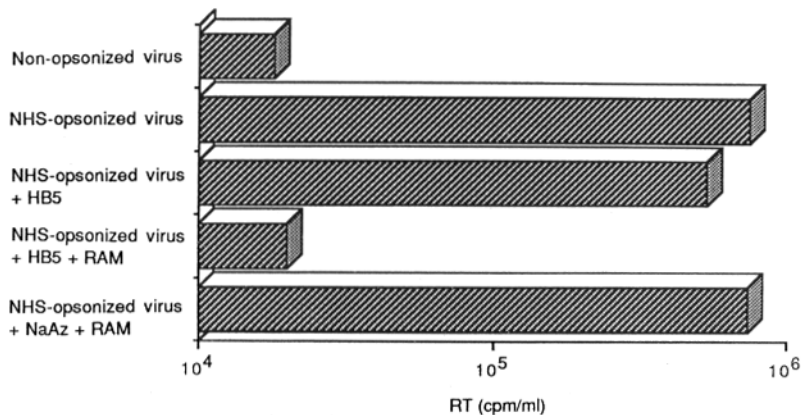
**Table 1.** Alternative Complement Pathway Dependency of the Enhancing Effect of HIV-seronegative Serum on Infection of MT2 Cells with HIV-1

Source of serum	RT activity
	<i>cpm</i> $\times 10^{-5}/\text{ml}$
Culture medium	0.18
NHS	7.6
NHS-EDTA	0.26
C2-deficient serum	7.8
Factor B-depleted serum	0.16

MT2 cells were infected with a fixed amount of the HTLV-RF strain of HIV-1 (MOI  $10^{-4}$ ) that had been preincubated with serum (25  $\mu\text{l}$ ) or with culture medium for 1 h at 37°C. RT activity was assessed at day 9 of the culture. NHS: serum from a healthy HIV-seronegative individual; NHS-EDTA: NHS that had been dialyzed against 10 mM EDTA; C2-deficient serum: serum from a homozygous C2 individual; factor B-depleted serum: NHS depleted of factor B of the alternative pathway.

that chelation of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  with EDTA or the use of factor B-depleted serum abolished the enhancing effect of serum on HIV infection of MT2 cells. C2-deficient serum exhibited a similar enhancing capacity as that of normal human serum, indicating that enhancement of infection was dependent on complement activation through the alternative pathway. Heat treatment of NHS (30 min at 56°C) also suppressed the enhancing effect of serum; normal guinea pig serum had no enhancing activity (data not shown).

*Complement-opsonized HIV-1 Infects MT2 Cells through Interaction with CR2 in a CD4-independent Fashion.* Staining with anti-C3 receptor mAbs demonstrated that MT2 cells only express the C3dg receptor, CR2 (CD21), and do not express CR1 (CD35) and CR3 (CD11b/CD18) complement receptor antigens (data not shown). The role of CR2 in medi-

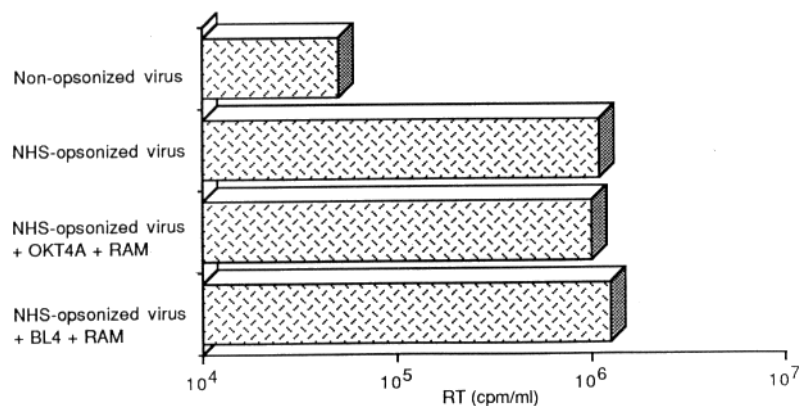


**Figure 3.** CR2 mediates infection of MT2 cells with complement-opsonized HIV-1. MT2 cells were infected with the HTLV-RF strain of HIV-1 (MOI  $10^{-4}$ ) that had been preincubated with 25  $\mu\text{l}$  of normal seronegative human serum as depicted in Fig. 1. Before infection, MT2 cells were incubated with saturating amounts of anti-CR2 antibody HB5 followed or not by rabbit F(ab')<sub>2</sub> anti-mouse Ig antibodies (RAM). RT activity was assessed at day 9 of culture. Nonopsonized virus: cells infected with virus in the absence of opsonization with serum; NHS-opsonized virus: cells infected with virus that had been preincubated with seronegative human serum; NHS-opsonized virus + HB5: cells preincubated with anti-CR2 mAb HB5 and infected with complement-opsonized virus; NHS-opsonized virus + HB5 + RAM: cells preincubated with anti-CR2 mAb HB5 crosslinked with RAM and infected with complement-opsonized virus; NHS-opsonized virus + NaAz + RAM: cells preincubated with NaAz (0.1%, i.e., at an equivalent concentration to that present in the HB5 mAb solution) and RAM, and infected with complement-opsonized virus.

ating the infection of MT2 cells with virus that had been opsonized with complement was demonstrated in the experiments depicted in Fig. 3. MT2 cells were cultured in the presence of suboptimal amounts of virus of the RF strain (MOI  $10^{-4}$ ) that had been pre-opsonized with seronegative serum. At day 9 of culture, infection with opsonized viral particles resulted in significant RT activity, whereas background levels of RT activity were observed in cultures infected with nonopsonized virus. Blocking of CR2 on MT2 cells with saturating amounts of the anti-CR2 mAb HB5 that had been cross-linked with F(ab')<sub>2</sub> anti-mouse Ig rabbit antibodies resulted in a background RT activity similar to that observed in control cultures infected with nonopsonized virus. The conditions used for blocking CR2 on MT2 cells were those that totally suppressed rosette formation of the CR2-expressing T cell line HPBALL with C3d-coated sheep erythrocytes (data not shown). No capping of CR2 antigen was observed on MT2 cells incubated with anti-CR2 and rabbit anti-mouse Ig antibodies (data not shown). Preincubation of MT2 cells with mAb HB5 without rabbit anti-mouse Ig antibodies suppressed RT activity of cells infected with complement-opsonized virus by ~30%.

Blocking experiments using anti-CD4 antibodies resulted in no inhibition of RT activity in cultures of MT2 cells infected with complement-opsonized virus (Fig. 4). The experiments were performed with saturating concentrations of mAb OKT4A, which binds to the gp120-binding site epitope on CD4, or with mAb BL4, which recognizes another epitope on CD4 independent of the binding site for gp120. Both mAbs OKT4A and BL4 were crosslinked with rabbit anti-mouse Ig antibodies. OKT4A and rabbit anti-mouse Ig F(ab')<sub>2</sub> were used in amounts that inhibited RT activity in cultures of MT2 cells infected with optimal amounts of virus (data not shown). Thus, infection of MT2 cells with preopsonized virus occurred through interaction with CR2, independently of the CD4 molecule.

**Complement-dependent Infection of PBMC with HIV-1.** PBMC from HIV-1-infected donors ( $10^6$  cells/well) were cocultured with an equal number of PHA-stimulated PBMC from an HIV-seronegative individual in the presence or ab-



**Table 2.** Enhancing Effect of HIV-seronegative Serum on Infection of Normal Human PBMC with HIV-1

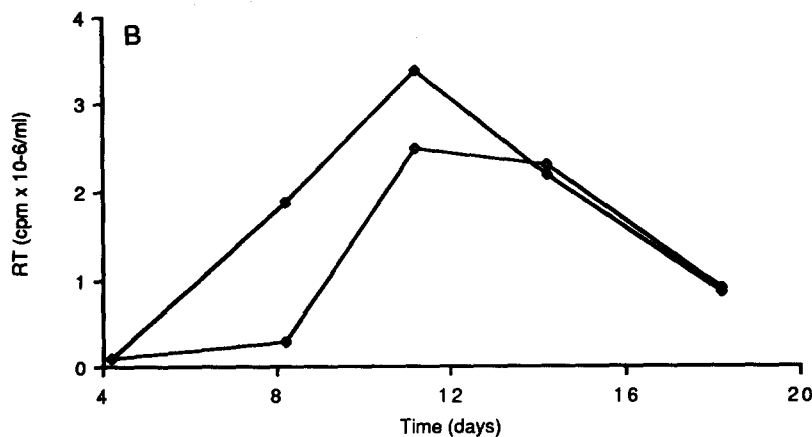
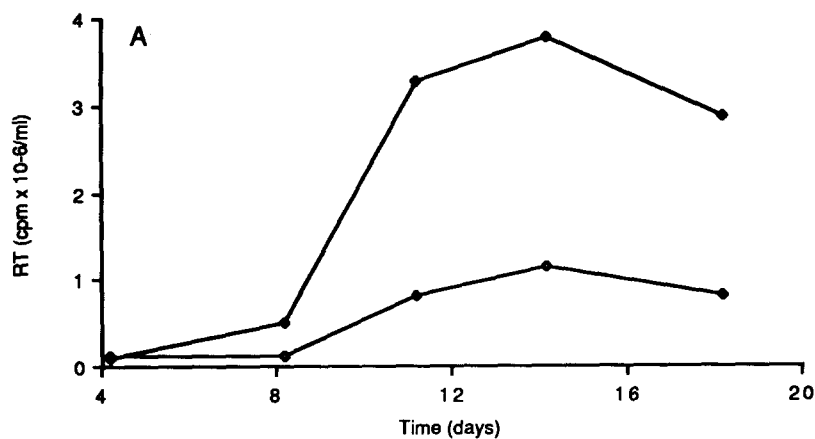
MOI	RT activity		Relative enhancement
	HIV opsonized with NHS	HIV opsonized with heat-treated NHS	
	<i>cpm × 10<sup>-4</sup>/ml</i>		
$2.5 \times 10^{-4}$	1.7	0.8	2.1
$2.5 \times 10^{-5}$	4.1	0.5	8.2
$2.5 \times 10^{-6}$	2.6	0.4	6.5

PBMC from a healthy seronegative donor were cultured with HTLV-RF that had been preincubated with NHS or heat-treated NHS and then stimulated with PHA (9 µg/ml) for 3 d before assessment of RT activity in culture supernatants.

sence of normal human seronegative serum (100 µl in 2.0 ml of final culture volume) for 18 d. Human serum was kept throughout the culture by adding fresh serum twice a week at the time when the culture medium was changed. The presence of serum resulted in up to 4- and 10-fold enhancement in RT activity in supernatants of coculture with cells from two HIV-infected donors (Fig. 5).

In another series of experiments, PHA-stimulated PBMC or purified peripheral blood T cells from four seronegative donors ( $10^6$  cells/well) were cultured with low amounts (MOI  $2.5 \times 10^{-5}$ ) of HTLV-RF that had been preopsonized or not with normal human seronegative serum. Depending on the donor, a 1.7–2.7-fold enhancement of RT activity was observed between days 3 and 9 in cultures of PBMC and of T cells infected with serum-opsonized virus as compared with cultures of cells treated with heat-inactivated serum. The enhancing effect of serum was abrogated if cells were pretreated with anti-CR2 mAb HB5 and F(ab')<sub>2</sub> anti-mouse rabbit antibodies (data not shown). Since PHA stimulation downregulates the expression of CR2 on human T

**Figure 4.** Lack of effect of anti-CD4 antibodies on infection of MT2 cells with complement-opsonized virus. MT2 cells were infected with the HTLV-RF strain of HIV-1 (MOI  $10^{-4}$ ) that had been preincubated with seronegative human serum as depicted in Fig. 1. Before infection, MT2 cells were incubated with saturating amounts of anti-CD4 mAb OKT4A or BL4 followed by rabbit F(ab')<sub>2</sub> anti-mouse Ig antibodies (RAM). RT activity was assessed at day 9 of culture. Nonopsonized virus: cells infected with virus in the absence of opsonization with serum; NHS-opsonized virus: cells infected with virus that had been preincubated with seronegative human serum; NHS-opsonized virus + OKT4A + RAM: cells preincubated with mAb OKT4A crosslinked with RAM and infected with complement-opsonized virus; NHS-opsonized virus + BL4 + RAM: cells preincubated with mAb BL4 crosslinked with RAM and infected with complement-opsonized virus.



**Figure 5.** Kinetics of enhancement by seronegative human serum of HIV infection of PBMC cocultured with infected PBMC. PHA-stimulated PBMC from a seronegative individual were cocultured with PBMC from two HIV-1-infected patients in the presence (◆) or absence (◇) of normal human seronegative serum.

cells, PBMC from one donor were infected with serum-opsonized or with nonopsonized HTLV-RF, washed, and then cultured in the presence of PHA for 3 d. As shown in Table 2, pretreatment of HIV with serum resulted in a 2.1–8.2-fold enhancement of RT activity, depending on the input of virus. Maximal enhancement of HIV infection was achieved at suboptimal amounts of HTLV-RF ( $\text{MOI } 2.5 \times 10^{-5}$ ).

### Discussion

The present study demonstrates that complement enhances infection of the human T cell line MT2 and of peripheral blood T lymphocytes with HIV-1, and may mediate alone the infection of cells with suboptimal amounts of virus. Infection of the cells with complement-opsonized virus occurred through an interaction of opsonized particles with the C3dg (CR2) receptor on target T cells in an antibody-independent process.

Preincubation of viral particles with normal seronegative human serum under conditions that allow complement activation resulted in enhanced infection of MT2 cells exposed to low amounts of virus. Enhancement of infection was greater when using the HTLV-RF strain of HIV-1, which is more effective in infecting MT2 cells than the HTLV-IIIB strain. No enhancement of infection had been observed by Robinson et al. (37) using the same target cells and strain of virus in the absence of anti-HIV antibodies; however, the relatively high amounts of virus that these authors used may have been above the threshold at which enhancement may be seen. Antibody-independent enhancement of HIV infection of MT2 cells has been observed when using desialylated virus (40). The latter phenomenon could be interpreted as being dependent on an increased deposition of C3 on the virus, through enhanced alternative complement pathway activation by desialylated viral particles (41). There is evidence that intact HIV-1 (30), the glycosylated recombinant gp160 envelope pro-

tein (Thieblemont N., N. Haeffner-Cavaillon, L. Weiss, F. Maillet, and M. D. Kazatchkine, manuscript submitted for publication), and HIV-infected cells (31) activate human complement in whole serum with subsequent binding of C3 fragments to the virus. Whereas infected cells activate the alternative pathway, intact HIV and the gp160 protein activate the classical pathway in an antibody-independent fashion. Our observations with C2 and with factor B-deficient serum indicate that complement activation by the infectious viral suspensions that we used occurred through the alternative pathway.

Fragments of C3 that become bound to complement activators may interact with at least three types of complement receptors for surface-fixed fragments of C3, termed CR1 (CD35), CR2 (CD21), and CR3 (CD11b, CD18) (32). The MT2 cell line only expresses the CR2 receptor that preferentially recognizes the C3dg fragment of C3, but may also bind C3b and iC3b. Blocking of CR2 by crosslinking the receptor with anti-CR2 mAb followed by a second anti-mouse Ig antibody totally suppressed RT activity in cultures of MT2 cells infected with HIV-1 that had been pre-opsonized with complement in the absence of antibody. We crosslinked the receptor on the cells because anti-CR2 mAb HB5 is not directed against the C3dg binding site on the CR2 molecule. The interaction of opsonized virus with CR2 was sufficient to allow penetration of the virus in MT2 cells and result in productive infection at day 9 of the culture, since blocking of CD4 with crosslinked anti-CD4 mAb OKT4A had no inhibitory effect on infection of the cells. Thus, serum-opsonized HIV-1 may penetrate into T cells through CR2 in an antibody- and CD4-independent fashion.

The role of CR2 in allowing infection of MT2 cells with opsonized HIV-1 was shown under experimental conditions using suboptimal amounts of virus and assessing RT levels at day 9 of culture of infected cells. It is possible that at higher concentrations of virus or if using a longer culture period, an additive or synergistic contribution of CR2 and CD4 in determining infection would have been observed. Such a synergistic effect of CR2 and CD4 has recently been reported

with EBV-infected B cells expressing CD4 and CR2, and virus that had been opsonized with both complement and anti-HIV antibodies (23, 24).

Approximately 40% of normal human peripheral blood T lymphocytes express low amounts of CR2 (35). We thus investigated the role of complement and CR2 in the infection of PBMC and of purified peripheral blood T lymphocytes in order to assess the physiologic relevance of the experiments using the MT2 cell line. The presence of normal seronegative serum in coculture of normal PHA-stimulated PBMC with cells from HIV-infected individuals resulted in an enhanced and earlier productive viral infection. In addition, opsonization of viral particles with seronegative serum resulted in a two- to threefold enhancement of RT activity in cultures of PHA-stimulated PBMC and purified peripheral blood T lymphocytes. The enhancing effect of serum opsonization of the virus was abrogated if cells were pretreated with crosslinked anti-CR2 mAb, as it had been observed with MT2 cells. The lower serum-dependent enhancement of infection that was observed with PBMC and peripheral blood T lymphocytes as compared with MT2 cells may be due to the low amounts of CR2 that are expressed by normal T lymphocytes and by the fact that PHA activation of human T lymphocytes is associated with a loss of expression of CR2 by the cells (35). The latter hypothesis was confirmed in experiments in which enhancement of infection of PBMC with opsonized HIV preceded PHA stimulation.

CD4 has been shown to be the primary receptor for HIV on human T cells (1, 2). The present observations indicate that opsonization of HIV-1 with the complement receptor CR2 in serum may result in enhanced infection of T cells and that the interaction of opsonized virus with complement may be sufficient for virus entry into target cells under conditions where suboptimal amounts of viral particles are present. The role of complement and of complement receptors on T cells and other target cells for the virus (42, 43) may thus be critical for viral propagation in infected patients carrying low amounts of virus and in patients at the time of primary infection with HIV-1.

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