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Cutting Edge: Productive HIV-1 Infection of Dendritic Cells via Complement Receptor Type 3 (CR3, CD11b/CD18)

Zsuzsa Bajtay,* Cornelia Speth,* Anna Erdei,† and Manfred P. Dierich1*

In the present study, we demonstrate that macrophage-tropic HIV-1 opsonized by complement and limited amounts of anti-HIV-IgG causes up to 10-fold higher productive infection of human monocyte-derived dendritic cells than HIV treated with medium or HIV opsonized by Ab only. Enhanced infection is completely abolished by a mAb specific for the ligand-binding site of CD11b (i.e., α-chain of complement receptor 3, receptor for iC3b), proving the importance of complement receptor 3 in this process. Inhibition of complement activation by EDTA also prevents enhanced infection, further demonstrating the role of complement in virus uptake and productive infection. Since HIV is, even in the absence of Abs, regularly opsonized by complement, most probably the above-described mechanism plays a role during in vivo primary infection. The Journal of Immunology, 2004, 173: 4775–4778.

Effectors of the complement system, one of the main components of natural immunity, include opsonization leading to enhanced phagocytosis and lysis of microbes. HIV is able to activate complement both in the absence and, even more so, in the presence of virus-specific Abs. Following binding of C1q to gp41 of HIV, the classical pathway of complement activation is initiated (1), while the lectin-dependent activation is initiated by the interaction of gp120 and gp41 with the mannann-binding lectin (2). Covalent binding of C3 fragments to the viral envelope allows binding of HIV to cells expressing receptors for C3b (complement receptor CR)2, 1, CD35), iC3b (CR3, CD11b/CD18; CR4, CD11c/CD18), and C3d, C3dg (CR2, CR2D1). HIV-1 infection of human lymphocytes and monocytes/macrophages is greatly enhanced by opsonization of the virus with complement (3–5).

In general, microorganisms coated with C3 fragments acquire the capacity for high binding to CR1–4 (3–7). CR3 and CR4 are members of the β2-integrin family, are expressed on several cell types, including phagocytic cells, and play an important role in phagocytosis of opsonized pathogens. It has been shown that dendritic cells (DCs) in normal human skin as well as monocyte-derived DCs (MDCs) differentiated in vitro express CD11b (8). Regarding the expression of CD11c, two major populations are present in the body: the CD11c+ myeloid DCs, and the CD11c– plasmacytoid DCs. CR3 and adhesion molecules are documented to facilitate viral entry into different target cells (9, 10).

CD14+ human monocytes can develop into MDCs in vitro by culturing in medium containing GM-CSF and IL-4. By day 5 in culture, the cells have the characteristics of immature DCs (imMDCs) and can be further induced to mature DCs by inflammatory stimulus such as LPS. Maturation rapidly induces DCs to express costimulatory molecules, such as CD80 and CD86, necessary for the activation of naive T cells (11). Targets of HIV during primary in vivo infection are macrophages and DCs, which are predominantly permissive for R5 viruses (12). imMDCs can be productively infected with R5 viruses and the virus produced by these cells is able to infect T cells. Mature DCs do not produce viral particles, still they are able to transmit both R5 and X4 viruses to T cells (13).

Our observation that complement opsonization of HIV causes a distinctively productive infection of DCs has not yet been documented. In this study, we report that CR3 plays a crucial role in the productive infection of imMDCs by C3-opsonized HIV.

Materials and Methods

Media, Abs, cytokines

Anti-human (hu) C3c, anti-huC3d, and anti-huIgG rabbit polyclonal Abs and FITC-conjugated goat anti-mouse Ig polyclonal Ab were obtained from DakoCytomation (Glostrup, Denmark). Mouse mAbs used in this work were as follows: anti-CD4, anti-CD86, anti-CD83, anti-CD14, anti-CCR5, anti-CD11c (BD Pharmingen, San Diego, CA) anti-CD18 (OKM-1), hybridoma supernatants and mouse IgG1 isotype control were used at 10 μg/ml. mAb TMG6-5 blocking anti-CD11b (14) was kindly provided by I. Ando (Biological Research Center, Szeged, Hungary).

DC cultures

MDCs were generated in vitro by culturing monocytes (15, 16). Briefly, PBMCs were obtained by the standard Ficoll-Hypaque (Pharmacia Biotech, Uppsala, Sweden) method. Monocytes were isolated by adherence using gelatin-coated petri dishes (17). The purity of the monocyte suspension was 90–95%, as judged by FACS analysis. Differentiation of monocytes into imMDCs was induced by culturing in RPMI 1640 medium (Invitrogen Life Technologies, Carlsbad, Sweden). 

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2 Abbreviations used in this paper: CR, complement receptor; DC, dendritic cell; MDC, monocyte-derived DC; imMDC, immature MDC; hu, human; rh, recombinant human; maMDC, mature MDC.
Grand Island, N.Y.) supplemented with 1600 U/ml recombinant human (rh) GM-CSF (Novartis, Vienna, Austria), and 1500 U/ml rhIL-4 (PromoCell, Heidelberg, Germany), 10% heat-inactivated FCS (Invitrogen Life Technologies), 2 mM l-glutamine, and 50 μg/ml gentamicin (Sigma-Aldrich, St. Louis, MO) in 24-well tissue culture plates (Costar, Cambridge, MA). Culture medium was refreshed at days 5 and 7 and supplemented with cytokines at days 2, 5, and 7. imMDCs were CD14+, CD80+, CD86+, CD83+, CD11b+, CD11c+, HLA-DR+, CD4low, and CCR5low (12, 18). imMDCs were used for virus binding and infectious experiments on day 7 of culture. Occasionally, in vitro maturation of MDCs was induced by 100 ng/ml LPS (Sigma-Aldrich).

HIV sources, p24 ELISA

The M-tropic HIV-1 primary isolate 92UG037 (subtypes/A, R5 tropism) was kindly provided by G. Stiegler (Institute of Applied Microbiology, Vienna, Austria). Virus stock was produced by PHA-activated PBMCs of healthy donors and maintained in RPMI 1640 supplemented with 10% FCS, 2 mM l-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. The virus was pelleted at 20,000 rpm by ultracentrifugation and stored at −80°C. For assessment of virus quantity, the p24 capture ELISA was used, developed at the Institute of Applied Microbiology in Vienna (19); it was performed as described earlier (18).

Opsonization of HIV, virus capture assay

Opsonized virus was prepared in the presence of anti-HIV IgG (purified from pooled sera of HIV+ patients) and normal human serum as complement source (HIVIgG+C) or in the presence of anti-HIV IgG alone (HIVIgG). Control samples were prepared in medium only (HIV). For opsonization, 1 μg/ml p24 HIV was incubated with 50 μg/ml anti-HIV IgG (HIVIgG) or with anti-HIV IgG and 10-fold diluted normal human serum (HIVIgG+C) for 1 h at 37°C. In some experiments, EDTA (20 mM) was used to inhibit complement activation (HIVIgG+C+EDTA). The opsonized virus was washed and pelleted by ultracentrifugation; aliquots were stored at −80°C and thawed once before the experiments. Opsonization was detected by a virus capture ELISA system. Preparation of nonopsonized HIV was incubated in wells coated with Abs specific for huIgG, or for complement fragments C3c and C3d, respectively. Captured HIV was lysed in detergent and transferred to anti-p24-coated plates. After incubation with anti-HIV IgG, the virus bound intensively to the anti-IgG coat. As a result of classical pathway activation of the complement system, strong C3 and low (if any) IgG deposition were detected in the HIVIgG+C samples, suggesting poor accessibility of the IgG on HIVIgG+C.

Binding of HIV to DC

To demonstrate binding of HIV, 5 × 10^4 imMDCs were incubated with 10 ng/ml p24 HIV, HIVIgG, HIVIgG+C, and HIVIgG+C+EDTA for 1 h at 37°C. Then nonbound virus was removed by washing two times with warm medium. The cells were lysed with 1% Nonidet P-40 and the amount of p24 Ag was determined from the HIVIgG+C samples, suggesting poor accessibility of the IgG on HIVIgG+C.

Infection of MDCs with HIV-1

To determine the infectivity of HIV, 5 × 10^4 imMDCs were incubated with 10 ng/ml p24 HIV, HIVIgG, HIVIgG+C, and HIVIgG+C+EDTA, respectively, for 8 h, then the cells were washed two times with warm medium and placed back into culture in 24-well plates for an additional 15 days. Twenty-five percent of medium was replaced with rhIL-4 and rhGM-CSF every third day. The amount of virus produced by the cells was estimated by p24 ELISA from the supernatants every other day.

Flow cytometry

The expression of surface molecules of cells was determined at day 7 of culture. Briefly, 5 × 10^4 cells were washed with 1% BSA-0.1% NaN3-PBS and incubated with Abs reacting with CD80, CD86, CD11b, CD11c, CD18, HLA-DR, CD14, CD4, and CCR5, followed by FITC-labeled anti-mouse IgG. As control, samples were labeled with isotype-matched control Abs. After staining, the cells were washed and fixed in PBS containing 1% Formalin and 0.1% NaN3 and analyzed by flow cytometry (FACScan; BD Biosciences, Mountain View, CA).

Results

Expression of CR3 during maturation of DCs

It has been demonstrated in earlier studies that DCs do not possess CR1 and CR2, while they express CR3 and CR4 (18, 20, 21). Since opsonization is known to influence viral infection (4, 5, 22) we aimed to clarify whether CR3, mediating the uptake of C-coated particles by several cell types, is involved in a similar process of MDCs. For these experiments we used the Ab TMG6-5, which interacts with the receptor’s ligand binding site. Fig. 1 shows that TMG6-5 binds to imMDCs and mature MDCs (maMDCs) in a similar fashion. It is also seen in Fig. 1 that the percentage of CR3+ cells is decreased in maMDCs.

![FIGURE 1. Surface expression of CD11b and CD18 of imMDCs and maMDCs at day 7. Shaded histograms represent the binding of CD11b- and CD18-specific Abs. Open histograms represent isotype-matched controls. Mean fluorescence intensity values are shown. The data are representative of seven independent experiments.](http://www.jimmunol.org/)

![FIGURE 2. Binding of nonopsonized and opsonized HIV to imMDCs. imMDCs at day 7 were incubated with HIV, HIVIgG, HIVIgG+C, and HIVIgG+C+EDTA for 1 h at 37°C. The concentration of p24 Ag was determined from the cell lysates after removing nonbound viruses. The data are representative of six independent experiments.](http://www.jimmunol.org/)
Effect of opsonization by complement on binding of HIV-1 to imMDCs

To investigate the binding of C-opsonized HIV, imMDCs were incubated with HIV, HIV\textsubscript{IgG}, HIV\textsubscript{IgG} + C, and HIV\textsubscript{IgG} + C + EDTA for control with HIV, HIV\textsubscript{IgG}, and HIV\textsubscript{IgG} + C + EDTA. After removing nonbound virus, cells were lysed and the amount of p24 Ag was determined by ELISA. Fig. 2 shows that HIV\textsubscript{IgG} + C binds more efficiently to imMDCs than HIV\textsubscript{IgG}. This enhancement was abolished when complement activation and opsonization were inhibited by 20 mM EDTA.

Effect of opsonization by complement on binding of HIV-1 to imMDCs

To investigate the binding of C-opsonized HIV, imMDCs were incubated with HIV\textsubscript{IgG} + C and for control with HIV, HIV\textsubscript{IgG}, and HIV\textsubscript{IgG} + C + EDTA. After removing nonbound virus, cells were lysed and the amount of p24 Ag was determined by ELISA. Fig. 2 shows that HIV\textsubscript{IgG} + C binds more efficiently to imMDCs than HIV\textsubscript{IgG}. This enhancement was abolished when complement activation and opsonization were inhibited by 20 mM EDTA.

Effect of opsonization of HIV by complement on infection of imMDCs

Infection of human monocytes and macrophages with HIV is greatly facilitated by opsonization of the virus with C3 fragments (5, 22). To test whether infection of imMDCs by HIV is influenced by opsonization, the cells were cultured with the HIV-1 primary isolate pretreated with IgG and complement. Culture supernatants were collected every other day, centrifuged, and the concentration of free virions was estimated by p24 ELISA. As demonstrated in Fig. 3, opsonization with HIV-specific IgG and human complement caused >10-fold enhancement in HIV production by MDCs, as compared with nonopsonized HIV or HIV opsonized with specific IgG only. Virus particles treated with serum in the presence of 20 mM EDTA caused low productive infection similar to the medium-treated control.

Treatment of imMDCs with CD11b-specific Ab or with proteinase K and their effect on infection by complement-opsonized HIV-1

To investigate the role of CR3 in the uptake of the opsonized virus, the cells were preincubated with mAb TMG6-5, which interacts with the ligand binding site of CD11b. As shown in Fig. 4A, the Ab reduced binding of HIV\textsubscript{IgG} + C to background level. Also, as seen in Fig. 4B, the productive infection of MDCs tested on day 14 was almost completely abolished by the CR3-specific Ab, while the isotype-matched control Ab showed no effect. To demonstrate that uptake of opsonized HIV was the key to productive infection, cells were treated with proteinase K before p24 measurement. As shown in Fig. 4A, this enzyme treatment resulted in a significant loss of HIV associated with MDCs.

Discussion

It is generally accepted that opsonization by complement enhances HIV uptake by CR-bearing cells, including MDCs. In this study, we show that opsonization of HIV with complement (and IgG) caused a slight increase in binding to MDCs but a 10-fold higher productive infection of imMDCs. This describes for the first time the crucial role of CR3 in this process.
In contrast to CR3, DC-specific ICAM-3-grabbing nonintegrin, another important attachment receptor for HIV on DCs, is known to bind HIV and to operate in transmission of the virus to CD4+ T cells (23); however, this does not induce productive infection of DCs. The key role of CR3 in efficient up-take of opsonized HIV by imMDCs was proven by inhibition with CD11b-specific mAbs. We also found that productive infection was strongly inhibited by anti-CD4 mAb (data not shown), suggesting that even in CR3-mediated enhancement of productive infection CD4 plays a critical role. In other studies, inhibition by anti-CR3 mAb of monocyte infection (18, 22) and partial inhibition by CD11b-specific Abs (10) of PBMC infection with HIV were observed.

Opsonization of HIV by complement occurs in human sebum by direct binding of C1q already in the absence of anti-HIV Abs (1, 2), but is much more pronounced after binding of Abs on the surface of HIV. Complement is also known to be activated by HIV upon direct binding of MBL; the activation of the alternative pathway in HIV-infected patients has also been observed (24). Other studies, however, show that intact HIV activates complement only in the presence of antiviral Abs (25, 26). Sensitivity to complement-mediated lysis is influenced by the actual composition of the viral membrane, as in vitro studies demonstrated that some primary isolates of HIV are highly resistant to complement-mediated lysis due to the incorporation of host cell-derived complement control proteins, including CD46, CD55, and CD59 (27, 28).

Since HIV particles in infected patients are early on coated with complement at the site of infection, imDCs could be the imDCs which are favorable for productive infection. Both aspects are subjects of future research.

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References


