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Follicular Dendritic Cells and the Persistence of HIV Infectivity: The Role of Antibodies and Fcγ Receptors

Beverly A. Smith-Franklin,1 Brandon F. Keele,2‡ John G. Tew,‡ Suzanne Gartner,§ Andras K. Szakal,‡ John D. Estes,‡ Tyler C. Thacker,‡ and Gregory F. Burton‡‡

Large quantities of HIV are found trapped on the surface of follicular dendritic cells (FDCs), and virus persists on these cells until they ultimately die. We recently found that FDCs maintain HIV infectivity for long periods in vivo and in vitro. Because FDCs trap Ags (and virus) in the form of immune complexes and are rich in FcγRs, we reasoned that Ab and FcγRs may be required for FDC-mediated maintenance of HIV infectivity. To investigate this hypothesis, HIV immune complexes were formed in vitro and incubated for increasing times with or without FDCs, after which the remaining infectious virus was determined by HIV-p24 production in rescue cultures. FDCs maintained HIV infectivity in vitro in a dose-dependent manner but required the presence of specific Ab for this activity regardless of whether laboratory-adapted or primary X4 and R5 isolates were tested. In addition, Abs against either virally or host-encoded proteins on the virion permitted FDC-mediated maintenance of HIV infectivity. We found that the addition of FDCs to HIV immune complexes at the onset of culture gave optimal maintenance of infectivity. Moreover, blocking FDC-FcγRs or killing the FDCs dramatically reduced their ability to preserve virus infectivity. Finally, FDCs appeared to decrease the spontaneous release of HIV-1 gp120, suggesting that FDC-virus interactions stabilize the virus particle, thus contributing to the maintenance of infectivity. Therefore, optimal maintenance of HIV infectivity requires both Ab against particle-associated determinants and FDC-FcγRs. The Journal of Immunology, 2002, 168: 2408–2414.

Follicular dendritic cells (FDCs)5 are found in the germinal centers of all secondary lymphoid tissues, where they trap and retain Ags in the form of immune complexes (ICs) formed with specific Ab and/or complement proteins (1–3). The ability of FDCs to trap and retain ICs is thought to be dependent on receptors for both complement proteins and the Fc portion of Iggs (4–8). FDC-trapped Ag remains in its native conformation, as evidenced by its continued recognition by specific Ab and, on isolation, its comigration with native Ag when applied to sizing columns (1, 3). Conventional soluble protein Ags are retained on FDCs in mice with a t1/2 of ~2 mo; however, Ags have been detected a full year after injection (1, 9, 10). Importantly, while Ag concentration declines with time, only a few picograms (~10) are needed to induce production of microgram levels of specific Ab (4, 11). Thus, even minute quantities of Ag on FDCs can induce potent specific Ab responses and maintain long-term IgG and IgE memory responses (1, 4, 10, 12).
gp120 from HIV, suggesting that Ab-FDC-FcγR interactions stabilize the virus particle, thus prolonging its infectious nature.

Materials and Methods

HIV-1 strains, cell lines, and detection of infectious HIV

The laboratory-adapted strains, HIV-1_HIV and HIV-1_MN, and primary HIV-1 isolates 91US054 (X4) and 92US714 (R5) (National Institutes of Health AIDS Research and Reference Reagent Program, Rockville, MD) were selected for use. HIV-1_HIV and HIV-1_MN were propagated in H9 cells grown in complete medium (CM) consisting of RPMI 1640 supplemented with HEPEs buffer (20 mM), 1% nonessential amino acid solution, t-glutamine (2 mM), 20% heat-inactivated, defined FBS (all from HyClone Laboratories, Logan, UT), and 50 μg/ml gentamycin (Life Technologies, Grand Island, NY). Primary virus was propagated in 3-day, PHA-activated PBL maintained in CM supplemented with 25 U/ml IL-2 (National Institutes of Health AIDS Research and Reference Reagent Program). Cell-free virus stocks were prepared from tissue culture supernatants harvested from acutely infected cells at peak reverse-transcriptase activity (generally 5–12 days postinfection). The virus-containing medium was pooled, filtered through a 0.45-μm membrane, aliquoted, and frozen in liquid nitrogen to provide a uniform stock of infectious virus. For quantitation, virus stocks were thawed and assayed for p24 concentration and/or reverse-transcriptase activity, and in some instances the infectious units were determined using tissue culture-infective dose (TCID50) analysis on H9 cells. Lab-adapted virus stocks typically contained 1 μg/ml p24, 1.5 × 10^3 cpml/mg reverse-transcriptase activity, and, when TCID50 analysis was performed, 1 × 10^5–10^6 TCID50/ml. Stocks of the X4 and R5 primary virus isolates respectively contained 144 ng/ml p24 and 308,000 cpml/mg reverse-transcriptase activity (isolate 91US054) and 194 ng/ml p24 and 443,000 cpml/mg reverse-transcriptase activity (isolate 92US714).

Isolation of human FDCs

Human FDCs were obtained from tonsils from HIV-uninfected patients, as previously described (23). Briefly, human tonsils were dissected into 3-mm squares and incubated at 37°C in CM containing collagenase (10 mg/ml) and DNase I (1% v/v). Following a 1-h digestion of tissue, cells were collected and placed in RPMI 1640 medium containing antibiotics, as indicated above, and heat-inactivated FBS (33% v/v). The remaining undigested tissue was incubated again in fresh RPMI 1640 medium containing the above enzyme mixture, and the cells were collected as before. After the second digestion, medium without enzymes was added to the remaining tissue, and the preparation was mixed by gentle pipetting to release cells remaining in the digested tissue. The collected cells were pooled, washed in fresh medium, resuspended in fresh RPMI 1640 medium, and then separated on a perfored 50% continuous Percoll (Amersham Biosciences, Piscataway, NJ) gradient. The low-density fraction was collected and washed free of Percoll and resuspended in CM. FDCs were further enriched using positive selection by MACS. Cells were incubated with primary Ab, HJ2 (mouse IgM Ab that binds human FDCs; kindly provided by M. Nahm, University of Alabama, Birmingham, AL), for 2 h with gentle agitation on ice. The cells were washed and then incubated with secondary Ab, rat anti-mouse IgM conjugated to magnetic microbeads (Miltenyi Biotec, Auburn, CA) for 60 min, followed by MACS. Enriched FDC preparations were found to be 60–90% pure by flow cytometry. Because FDCs are radiation resistant, FDC preparations were gamma irradiated (3000 rad) before incubation with HIV to minimize the ability of any contaminating cells to support HIV infection.

Maintenance of infectious HIV in vitro

HIV-ICs were formed by incubating HIV-1 (20–100 μl viral stock based on the concentration of infectious virus in the individual preparation) with the indicated Abs (200 μg, unless otherwise specified) for 1–2 h at 37°C in CM. The Abs used were as follows: murine IgG, nonneutralizing, anti-gp41 (Chesie 8; National Institutes of Health AIDS Research and Reference Reagent Program), murine IgG, anti-HLA-DR (DP, DQ), and DQ (IWA12, American Type Culture Collection, Manassas, VA), and mouse or rat control IgGs (ChromPure; Jackson ImmunoResearch Laboratories, West Grove, PA). FDCs (10,000, unless otherwise specified) were added immediately after the ICs were formed, and the mixture was then incubated for increasing times at 37°C to assess the length of time that infectious virus remained for the different conditions. In one study, to determine whether FDCs needed to be viable for maintenance of HIV infectivity, the cells were subjected to fixation using phosphate-buffered paraformaldehyde (4%; 4 h on ice) before use.

Rescue of infectious virus

Rescue of infectious virus remaining after increasing periods of culture in FDCs was performed by adding 1 × 10^5 H9 cells (or 2 × 10^7 3-day PHA-activated, IL-2 (25 U/ml)-treated PBL for primary virus isolates) to the virus preparations and culturing for an additional 2 days (6 days with primary cells) to permit infection. Culture supernatants were then assayed for p24 production by a kinetic Ag-capture ELISA (Beckman Coulter, Palo Alto, CA), according to the manufacturer’s instructions. Production of HIV-1 p24 was determined by subtracting the input p24 from the total p24 concentration detected after culture.

Blocking FcγRs on murine FDCs in vitro

Murine FDCs were obtained using a protocol similar to that used for human FDCs with minor modifications, as previously described (29). Murine FDCs were used because of the ability of the mAb, 2.4G2 (rat IgG2b anti-murine FcγRII/RIII), to block FcγRs present on FDCs. Isolated FDCs were cultured for 2 h with either control Ab (rat IgG) or 2.4G2. FDCs were then added to cultures of HIV only or HIV-ICs formed with anti-gp41 (Chesie 8) for an additional 2 days, and infectious virus present was determined, as described above.

Maintenance of HIV infectivity with FcγR-transfected CHO cells

FcγRII-transfected Chinese hamster ovary (CHO) cells (kindly provided by D. Conrad, Virginia Commonwealth University, Richmond, VA) were examined for their ability to maintain HIV infectivity. Before their use, cells were subjected to gamma irradiation (10,000 rad) to block their ability to proliferate. These cells or FDCs (as controls) were then cultured with HIV-ICs formed with either anti-gp41 or anti-MHC II (HLA-DR, DP, and DQ) for 12 days, during which time virus rescue cultures were performed, as described above.

Detection of gp120 shedding

HIV-1_HIV and HIV-1_MN alone or in ICs formed with anti-gp41 (Chesie 8) were cultured for 2 days, as described above. Cultures were then centrifuged at 100,000 × g to pellet cells and intact virions, thus providing separation from spontaneously released soluble gp120. Supernatant fluid was then collected and assayed for released gp120 by Ag-capture ELISA (ImmunoDiagnostics, Bedford, MA), according to the manufacturer’s instructions. In addition, replicate cultures were assessed to determine the infectious virus remaining, as described above.

Results

FDCs mediate the protection of HIV in vitro in a dose-dependent manner and require the presence of specific Ab

Previously, we found that FDCs could maintain the infectious nature of HIV for at least 9 mo in vivo and 25 days in vitro (23). In this study, we sought to extend this work to assess the contributions of virus-specific Ab and FDC-FcγRs in the maintenance of HIV infectivity by FDCs. For this work, we used our in vitro culture system that was amenable to manipulation. We first sought to establish whether FDC maintenance of virus infectivity was dose dependent by incubating HIV-ICs in the presence of decreasing numbers of FDCs (Fig. 1). Similar to our previous work (23), after 10 days, HIV alone or HIV-ICs retained only minimal ability to cause infection of H9 target cells (<1 ng HIV p24 produced). In contrast, the addition of 10,000 or 1,000 FDCs at the onset of culture resulted in the maintenance of viral infectivity, as indicated by the production of >20 ng HIV p24. Remarkably, even as few as 100 FDCs maintained sufficient infectious virus to produce HIV p24 levels that were well above background.

Because it has been reported that FDC-HIV interactions can occur in the absence of HIV-specific Ab (30), we sought to determine whether FDCs could provide maintenance of HIV_in vitro infectivity in the absence of Ab (Fig. 2). Surprisingly, the addition of FDCs in the absence of Ab resulted in no ability to maintain virus in an infectious state. We also sought to determine whether FDCs had to be viable to provide protection of viral infectivity. To test
this, FDCs were subjected to fixation and then cultured with HIV-ICs. These FDCs failed to provide maintenance of infectivity (<1.2 ng HIV p24 vs >48 ng with viable FDCs), consistent with the concept that a portion of the FDC-virus interaction leading to maintenance of viral infectivity requires viable FDCs (data not shown). To ensure that FDC-mediated protection of infectivity was not limited to HIV-1 

FIGURE 1. FDC maintenance of HIV_{11IB} infectivity in vitro is dose dependent. HIV alone or in ICs (HIV-ICs) formed with anti-gp41 (Chessie 8) were incubated at 37°C for 10 days ± the indicated number of FDCs. To assess the ability of virus to cause infection, H9 target cells (100,000) were added for an additional 2 days, and the quantity of HIV p24 produced (total p24 concentration - input virus p24) in culture supernatant fluid was determined by ELISA. Note that HIV alone or in the form of ICs had only minimal infectivity remaining after 10 days. In contrast, the same HIV-ICs incubated in the presence of FDCs have significant infectivity remaining and were directly proportional to the number of FDCs present. Data are expressed as the mean ± SEM of three experiments.

We postulated that the presence of Ab in our FDC cultures might have provided a “handle” whereby the FDCs could bind the HIV and as a consequence maintain its infectivity. If this were so, we reasoned that Ab to a nonviral encoded protein, present on the virion, would also allow the FDC to bind HIV and maintain virus infectivity. Because HIV acquires host proteins, including MHC class II molecules, in the process of budding from the host cell, and because the H9 cells used to propagate HIV_{11IB} are rich in MHC II proteins (31), we sought to determine whether anti-MHC II (anti-HLA-DR, DP, DQ) would also allow FDC maintenance of virus infectivity. We first confirmed that the H9-propagated virus preparations reacted with our anti-HLA-DR, DP, DQ Ab by performing nonreducing SDS-PAGE of viral lysates, followed by Western blotting using the MHC class II-specific Ab as a probe (data not shown). HIV-ICs were then formed using either anti-gp41 or anti-MHC II, as described, and cultured with FDCs for increasing periods of time (Fig. 4). FDCs were able to maintain HIV infectivity when either Ab to HIV gp41 or human MHC II was used to form ICs. Although some decreased maintenance of virus infectivity was observed when anti-MHC II was used in place of anti-gp41, significant production of HIV p24 (nanogram levels) occurred using either specificity of Ab to form the HIV-ICs. To determine whether FDC-HIV-IC interactions needed to occur at the time of IC formation for optimal preservation of infectivity, we formed HIV-ICs as before and added FDCs immediately or after increasing time intervals, after which we assessed remaining virus infectivity (Fig. 5). Although the addition of FDCs after 7 days of culture appeared to have some effect on maintaining virus infectivity, optimal maintenance occurred when FDCs were added immediately after the formation of the viral ICs, as would likely occur in vivo.

FIGURE 2. Specific Ab was needed to maintain HIV_{11IB} infectivity. HIV and HIV-ICs were added to FDCs and cultured for 2 days. H9 target cells were then added, and the culture was continued for an additional 2 days. As before, infectious HIV was detected by its ability to infect target cells and produce p24 during a 2-day culture period. Note that little, if any, infectious virus was rescued when FDCs were cultured without specific Ab. Data are expressed as the mean ± SEM.
Murine FDCs were incubated with 2.4G2 or control rat IgG/H9253 FDCs and the rat, anti-murine Fc

Optimal FDC maintenance of HIV infectivity occurs when FIGURE 5. Data are expressed as the mean reduction as a measure of the ability of remaining virus to cause infection.

Fluid from the cell cultures was then collected and tested for p24 Ag production after 5, 10, or 12 days. Supernatant fluid from the cell cultures was then collected and tested for p24 Ag production as a measure of the ability of remaining virus to cause infection. Data are expressed as the mean ± SEM.

FIGURE 5. Optimal FDC maintenance of HIV infectivity occurs when FDCs and HIV-ICs are brought together shortly after IC formation. To test whether FDCs need to be present at the onset of culture with HIV-ICs (anti-gp41) to maintain infectivity, FDCs were added to HIV-ICs either immediately or at day 7, 10, or 14 after IC formation. HIV-ICs alone acted as a control. After the addition of FDCs, cultures remained at 37°C for 2 days, then target cells were added for an additional 2 days. Supernatant fluid from the cell cultures was then collected and tested for p24 Ag production as a measure of the ability of remaining virus to cause infection. Data are expressed as the mean ± SEM.

FDC-FcγRs are necessary, but not sufficient, for maintenance of HIV infectivity

FDCs bear high levels of FcγRII that appear important in their ability to trap and retain Ag-Ab complexes (4, 5, 8, 28). HIV-ICs can bind to FDCs through the interaction of the Fc portion of Ig present in the IC and FcγRs (FcγRII) located on FDCs (18, 22, 23). We therefore examined the importance of these receptors on FDCs in maintaining viral infectivity. Because potent blocking Abs are not available for FcγRs on human FDCs, we used murine FDCs and the rat, anti-murine FcγRII/RIII-blocking mAb, 2.4G2 (32). Murine FDCs were incubated with 2.4G2 or control rat IgG before and during culture with HIV-ICs, and rescue experiments were performed as described before (Fig. 6). Cultures containing 2.4G2, but not control IgG, showed markedly reduced maintenance of HIV infectivity. Interestingly, treatment with blocking mAb to FDC-FcγRII/RIII resulted in an 80% reduction of virus infectivity, but did not completely abrogate this activity, suggesting that other features of FDC biology may contribute to the maintenance of HIV infectivity. Also of note, the addition of 2.4G2- or control IgG-treated FDCs to HIV alone again failed to provide any maintenance of infectivity, confirming the earlier results indicating that specific Ab was required for FDC maintenance of HIV infectivity (Figs. 2 and 3).

We hypothesized that if FDC-FcγR-HIV-IC interactions were important in maintaining virus infectivity, then other cells bearing FcγR might also help maintain HIV in an infectious form. To test this hypothesis, we used CHO cells that had been transfected with FcγRI, reasoning that the presence of this receptor would optimize HIV-IC binding. These FcγRI-transfected cells (or human FDCs as a control) were cultured with HIV-ICs formed with anti-gp41 or anti-MHC II and incubated for 5, 10, or 12 days, followed by the addition of H9 cells for virus rescue (Fig. 7). Examination of the cultures revealed that HIV-ICs incubated without cells lost all infectivity after 5 days of incubation; however, in the presence of the transfected CHO cells or FDCs, infectious virus could be detected throughout day 12. As observed previously, slightly higher levels of p24 were detected in cultures containing anti-gp41 than in those with anti-MHC. Although there were some differences in maintaining infectious virus between the FcγRII-transfected CHO cells and FDCs, the observation that other cells bearing FcγRs can also maintain HIV infectivity supports the hypothesis that anchoring HIV-ICs via FcγRs appears important in maintaining the infectivity of the virus.

FDC-Ab interactions decrease the spontaneous loss of HIV gp120

One of the ways in which HIV_inh has been shown to lose infectivity in culture is attributed to the spontaneous loss of gp120
which is noncovalently associated with gp41) from the virion (33). We therefore reasoned that perhaps FDC-HIV-IC interactions resulted in an inhibition of gp120 shedding, thereby preserving the infectious nature of the virus. To test this postulate, we cultured HIV-1 IIIB and HIV-1 MN, a similar T tropic laboratory-adapted virus, alone or with anti-gp41/H11006 FDCs, and assessed the amount of HIV gp120 released into the tissue culture fluid (Fig. 8A). In parallel cultures, we also determined the ability of the remaining virus to cause infection of H9 target cells (Fig. 8B). Both strains of HIV spontaneously lost gp120 when cultured in the absence of Ab and FDCs. Importantly, the addition of FDCs to HIV-ICs reduced the amount of gp120 present in the tissue culture medium by $\frac{1}{2}$ with both strains of virus. Furthermore, the ability of the remaining virus to cause infection in rescue cultures inversely correlated with the amount of gp120 released into the culture medium, consistent with the hypothesis that FDCs reduce gp120 shedding, thus maintaining the ability of HIV to interact with CD4 and coreceptors to cause infection.

Discussion

Previously, we reported that FDCs maintain HIV infectivity both in vivo and in vitro (23). In the present study, we demonstrated the need for specific Ab and FcγRs in this process. Our studies suggest that Ab can play an important role in interactions between virus and FDCs. The role of specific Ab in FDC trapping and retention of HIV has been somewhat controversial (23, 30, 34, 35). We have used specific Ab as a means of localizing both Ags and HIV on FDCs both in vitro and in vivo (22, 23). In one preliminary study using another retrovirus (LP-BM5) that could replicate in mice, we found that FDC trapping was much greater when specific Ab was present (E. Griffiths and G. F. Burton, unpublished observations). Likewise, Ab appears to play an important role in follicular trapping of conventional Ags and the subsequent induction and maintenance of specific recall immune responses (1, 3, 8, 36). In contrast to our findings on FDC trapping, Fujiwara et al. (30) observed that Ab did not appear to be needed for FDC binding of HIV, but rather that this was mediated by interactions between CD54 and CD11a on HIV particles and FDCs. Others have found that complement proteins and their degradation products, particularly C3, appear to be important in FDC trapping of HIV (34, 35).

![Figure 7](http://www.jimmunol.org/)

**FIGURE 7.** FcyRI-transfected CHO cells were able to maintain the infectivity of HIV-ICs. HIV-ICs formed with anti-gp41 or anti-MHC II were added to 10,000 FDCs or gamma-irradiated, FcyRI-transfected CHO cells. After 5, 10, and 12 days of incubation, H9 target cells were added and cultured for an additional 2 days to detect infectious virus as assessed by p24 production. Note that the ability of viral ICs to infect target cells was lost by day 5 in the absence of FcγR-bearing cells, whereas their presence resulted in maintenance of infectivity for as long as 12 days. One of three similar experiments is shown with the data expressed as the mean ± SEM.

![Figure 8](http://www.jimmunol.org/)

**FIGURE 8.** FDC-Ab interactions inhibited the spontaneous release of gp120 from the virion, and this correlated with increased HIV infectivity. A. HIV-1 IIIB and HIV-1 MN were cultured with or without anti-gp41 for 2 days ± FDCs, and the amount of gp120 released into the tissue culture medium was assayed by ELISA. B. The ability of the remaining virus in parallel cultures to cause infection was assessed by HIV p24 production in H9 rescue cultures.
our studies did not specifically address the role of either adhesion molecules or complement proteins in FDC trapping of HIV, there was some FDC activity when FcR were blocked, and this may relate to other adhesion molecules. Nevertheless, the vast majority of the activity depended on Ab and FcR. If FDC trapping of HIV occurred in the absence of Ab in our system, then the failure to provide substantial protection of infectivity suggests that there may be fundamental differences in the outcomes of virus-FDC association, depending on the molecules involved in the process.

In addition to the necessity of specific Ab, we also identified an important role for FcγRs in the maintenance of HIV infectivity. Recently, it was reported that FcγRII plays an important role in FDC Ag trapping (8). Our studies also implicated this FcγR in FDC-HIV interactions. We found that blocking FcγRII/RIII on FDCs reduced their ability to maintain infectious virus. Significantly, this treatment did not completely abrogate the ability of FDCs to provide some maintenance of infectivity of HIV-ICs, suggesting that FDCs may make other contributions in addition to providing a rich source of FcγRII for trapping of HIV-ICs. In further support of this concept, we found that CHO cells transfected with FcγRI also maintained HIV infectivity over a 12-day period. Although some differences were observed in the amount of HIV p24 produced in rescue cultures of HIV-ICs incubated in the presence of the FcγR-bearing CHO cells vs the FDCs, these most likely relate to the presence of different FcγRs on the two cell types, different receptor densities, and the different cellular interactions that could occur between the cell types and the H9 target cells. Even with these differences, we interpret these data, coupled with the observed decrease in the maintenance of virus infectivity when FDC-FcγRs were blocked to support the importance of FcγRs in the maintenance of HIV infectivity.

Other FDC contributions that may play roles in maintaining HIV infectivity include such features as providing a reducing environment, because these cells are rich in thiol compounds that could be important in providing for optimal germinal center development (37). Perhaps the presence of thiol groups on FDCs may play a role in stabilizing HIV-ICs, thereby making them more resistant to degradation. FDCs also have extensive interdigitating dendrites that join with each other to form an elaborate reticulum bearing Ab-covered HIV. In this setting, it may be difficult for phagocytic cells to capture and eliminate viral particles. In support of this hypothesis, Ags trapped on FDCs appear to be surrounded by FDC dendrites during much of the germinal center reaction, thus sequestering these ICs from surrounding immune cells and presumably prolonging retention of the Ags (2, 38). In addition to the ability of FDCs to provide a protective environment, FDCs also contribute signals to lymphocytes that increase their state of activation (29, 39, 40). One potential consequence of this signaling is to increase the susceptibility of CD4 lymphocytes to HIV infection and replication (21). Thus, FDCs may contribute to maintaining virus infectivity in a number of ways in addition to those mediated by Ab and FcγRs.

Mechanistically, our work suggests that the spontaneous loss of gp120 from HIV, which has been noted previously as a contributing factor to the loss of infectivity (33), was reduced in the presence of FDCs, and that virus in cultures in which this loss was minimal preserved a greater ability to cause infection than when gp120 shedding was higher. However, our study did not define how the FDCs inhibited gp120 shedding. We envision that FDCs bind HIV-ICs on multiple dendritic processes using a number of different FcγRs, and that these interactions may physically restrain the virus particle such that gp120 dissociation is inhibited. In support of this hypothesis, we found that Ab that could bind to virion proteins, other than envelope glycoproteins, would still allow FDC-HIV interactions that could prevent the loss of gp120 and thereby maintain the infectious nature of the virus. In our system using Ab directed against HLA-DR, DP, and DQ, we envision that the binding of this Ab to MHC II molecules present on the surface of viral particles then permits particle association with FDCs. This association, we reason, may inhibit gp120 shedding by creating isolated regions in which the envelope glycoproteins are trapped between interacting dendritic processes. In this manner, the FDC would physically block the spontaneous release of gp120. Alternatively, it may be that the presence of Ab on the virion envelope coupled with binding to FDCs sterically hinders or in some other manner prevents the loss of gp120. Whatever the mechanism(s) is, the interaction of HIV-ICs with FDCs maintains the infectious capability of the virus and correlates with a reduced loss of the HIV gp120.

Shortly after introduction of Ag into immune animals, virus-Ab complexes form and are transported within seconds to draining secondary lymphoid tissues (38, 41). These Ags rapidly become trapped on FDCs and remain for many months. In HIV disease, FDC trapping of virus occurs shortly after infection. This virus trapping by FDCs early in the disease course may be advantageous for the virus, because at least in vitro, rapid association of virus ICs and FDCs led to optimal preservation of HIV infectivity. This implies that early seeding of the lymphoid organs with HIV and subsequent trapping on FDCs may increase the stability of the virus and hence its ability to cause infection. FDC-trapped virus could also be potentially replenished, as small amounts of virus replication are thought to occur even during HAART (24, 42). Because virus is trapped early, in many cases before the institution of HAART, mutants capable of avoiding selective pressure may be trapped on FDCs, and these archived quasi-species could remain for long periods in an infectious form awaiting an opportunity to cause infection, as might occur under drug-induced selective pressure. Thus, from the perspective of the virus, localization on FDCs may be highly advantageous, not only because of close proximity to activated target cells, but also because the FDC represents a sheltered environment that prevents the virus from being destroyed or degraded.

The microenvironment of the germinal center creates an ideal location for HIV to cause and maintain infection. FDCs trap large quantities of virus and retain them for long periods in an infectious form. HIV is trapped early in disease and remains until the FDCs are ultimately destroyed. FDCs also interact intimately with surrounding lymphocytes including CD4 T cells, thus affording an ideal opportunity for virus to cause infection. Furthermore, FDCs contribute signals that result in increased activation of surrounding cells. In addition, FDCs permit infection to occur even in the presence of high levels of neutralizing Ab that would otherwise prevent this. Finally, it is well known that cell-associated transmission of retroviruses is far more efficient than free virion transmission (43). An understanding of FDC-HIV interactions may be essential in developing successful intervention strategies that target this major, yet little understood HIV reservoir.

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