Role for CD21 in the Establishment of an Extracellular HIV Reservoir in Lymphoid Tissues[1]

Jason Ho,[2]* Susan Moir,[2,3]* Liudmila Kulik,[†] Angela Malaspina,* Eileen T. Donoghue,* Natalie J. Miller,* Wei Wang,* Tae-Wook Chun,* Anthony S. Fauci,* and V. Michael Holers[†]

Follicular dendritic cells (FDC) represent a major extracellular reservoir for HIV. A better understanding of the mechanisms of virion attachment to FDC may offer new avenues for reducing viral burdens in infected individuals. We used a murine model to investigate the establishment of extracellular HIV reservoirs in lymph nodes (LN). Consistent with findings in human tissues, CD21 was required for trapping of HIV to LN cells, as evidenced by significantly reduced virion binding when mice were pretreated with a C3 ligand-blocking anti-CD21 mAb and absence of virion trapping in CD21 knockout mice. Also consistent with findings in human tissues, the majority of HIV virions were associated with the FDC-enriched fraction of LN cell preparations. Somewhat surprisingly, HIV-specific Abs were not essential for HIV binding to LN cells, indicating that seeding of the FDC reservoir may begin shortly after infection and before the development of HIV-specific Abs. Finally, the virion-displacing potential for anti-CD21 mAbs was investigated. Treatment of mice with anti-CD21 mAbs several days after injection of HIV significantly reduced HIV bound to LN cells. Our findings demonstrate a critical role for CD21 in HIV trapping by LN cells and suggest a new therapeutic avenue for reducing HIV reservoirs. *The Journal of Immunology,* **2007, 178: 6968–6974.**

During the chronic phase of HIV infection, the bulk of the virus resides in secondary lymphoid tissues where follicular dendritic cells (FDC)[4] within follicles trap large quantities of replication-competent virions in the form of immune complexes (1–3). FDC-bound HIV is thought to be a major source of virus for replication in activated $CD4^+$ T cells, the main cell type responsible for virus production (4, 5). FDC also play an important role in humoral immune responses by providing costimulatory and survival signals to B cells undergoing germinal center reactions (6, 7). The high levels of viremia and extensive hyperplasia observed in lymphoid tissues of HIV-infected viremic individuals may impede the function of FDC (8, 9), and as such, contribute to the inability of these individuals to mount appropriate Ab responses to specific Ags (10–13).

Studies on mechanisms of HIV trapping and dissemination associated with FDC are notoriously difficult to conduct due to difficulties in isolating highly purified intact FDC. Nonetheless, in situ analyses conducted by Kacani et al. (14) on tissues isolated from HIV-infected viremic patients established a prominent role for complement receptor 2, CR2 or CD21, in the trapping of HIV by cells residing in follicles. These findings were consistent with our findings on B cells isolated from HIV viremic patients in which replication-competent HIV virions, in the form of immune complexes, were found to be bound to the B cells through interactions between complement breakdown product C3 complexed to virions and complement receptor CD21 expressed on B cells (15). Furthermore, by isolating lymphoid tissue-derived B cells to high purity, we demonstrated that the extracellular virus bound to B cells was closely related in nucleotide sequence to virus actively replicating in $CD4^+$ T cells isolated from the same tissue (16), suggesting transfer of infectious virus from B to T cells. These data not only suggest virologic cross-talk between B cell and T cells, but by extension to in situ data (14) also suggest a similar role for FDC, which in contrast to B cells, may represent a more stable network for trapping HIV and maintaining its infectivity (3, 17).

In previous studies, we and others have demonstrated that certain anti-CD21 mAbs have the property of not only blocking ligand binding to CD21, but also being effective at displacing bound ligand (14, 15, 18). These properties were demonstrated in the context of C3-complexed HIV both in vitro and ex vivo with B cells or tissues isolated from HIV-infected patients. Displacement of HIV from extracellular reservoirs could represent a new therapeutic approach to reducing a reservoir that is insensitive to current antiretroviral drugs. In the present study, we expand on an in vivo mouse model that was established for investigating the longevity of FDC-bound HIV (17) to evaluate the role of CD21 in the establishment of an extracellular HIV reservoir and as a target for virion displacement.

Materials and Methods

Reagents for mice injections

Hybridomas of mouse IgG1, anti-HIV envelope mAb Chessie 8 (19) and mAb 902 (20), were obtained through the AIDS Research and Reference Reagent Program (National Institutes of Health, Germantown, MD) and propagated as indicated. An ascites source of mouse IgG2a, anti-HIV envelope mAb 178.1 (21), was obtained through the National Institute for Biological Standards and Control Centralized facility for AIDS Reagents. The hybridoma propagation medium containing mAbs Chessie 8 and 902 were used directly for injection following clarification and passage over a 0.22-μm filter. The supernatant of a nonsecreting hybridoma was used as

*Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892; and [†]Department of Medicine and Immunology, University of Colorado Health Sciences Center, Denver, CO 80262

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[2] J.H. and S.M. contributed equally to this work.

[3] Address correspondence and reprint requests to Dr. Susan Moir, Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Building 10, Room 6A02, 10 Center Drive, Bethesda, MD 20892. E-mail address: smoir@niaid.nih.gov

[4] Abbreviations used in this paper: FDC, follicular dendritic cell; LN, lymph node.

control. Alternatively, IgG in the hybridoma and ascites was purified using protein G (Amersham Biosciences) and eluted into PBS. Endotoxin was removed with a Detoxi-gel endotoxin removing gel (Pierce). All Ig concentrations were adjusted to 60 μg/ml with hybridoma propagation medium or PBS. The replication-defective HIV isolate HIV-1 MC99IIIBΔTat-Rev and the corresponding CEM-TART cell line used to propagate the virus (22) were obtained through the AIDS Research and Reference Reagent Program. The virus preparation was clarified, filtered, and concentrated by ultrafiltration to 4 μg/ml as determined by ELISA for HIV-1 p24 (Beckman Coulter). Hybridoma of rat anti-CD21 mAb 7G6 originally provided by Dr. T. Kinoshita (Osaka University, Osaka, Japan), was grown in DMEM with 10% FCS and supplements, purified using protein G, and eluted into PBS. Isotype control Ab reconstituted in PBS was obtained (Sigma-Aldrich). Endotoxin was removed from both mAb 7G6 and its isotype control. Ig concentrations were adjusted to 10 mg/ml with PBS.

Mice and mice procedures

C57BL/6 mice were purchased from The Jackson Laboratory. Mice deficient in CD21/CD35 ($Cr2^{-/-}$) were generated on a C57BL/6 background as previously described (23). All procedures were performed in accordance with animal study protocols approved by the animal study committee of the National Institute of Allergy and Infectious Diseases. Male mice 6–8 wk of age were used in all experiments. In experiments designed to test the effects of Abs on HIV trapping (see Fig. 1, *left*), mice were i.p. injected with 100 μl of the indicated Abs or controls 1 day before the injection of HIV. In experiments designed to test ligand displacement (see Fig. 1, *right*), mice were injected with HIV followed by injection of anti-CD21 mAb or isotype control at day 4–6, with retrieval of lymph node (LN) cells 2 days later. Injections of HIV preparation consisted of 50 μl in one footpad and 100 μl in the tailbase, for a total of 600 ng of HIV p24 or ~5×10^9 HIV RNA copies per mouse. To retrieve draining LN, mice were sacrificed and appropriate popliteal and surface inguinal LN were collected and transferred to dissection medium (RPMI 1640 medium containing 20 mM HEPES and 10 U/ml RNase inhibitor (Roche)). Single-cell suspensions were obtained by gentle mechanical disruption using plastic pestles (Kimble/Kontes), followed by enzymatic digestion for 15 min at 37°C with 450 U/ml collagenase type 4 and 10 U/ml DNase I (Worthington Biochemical) and constant stirring. When necessary, additional 15-min incubations with fresh enzymes were performed.

FDC enrichment and HIV p24 measurements in lysates

FDC were enriched from LN as described (24). Briefly, popliteal LN were collected from six mice at 5 days after HIV injection. Single-cell LN suspensions were prepared by enzymatic digestion as described. Every 15 min, single cells were collected and fresh enzyme was added until digestion was complete. Recovered cells were incubated at a concentration of 2×10^7 cells/ml with 0.6 μg/ml anti-FDC mAb FDC-M1 (BD Biosciences) for 60 min at 4°C. FDC-M1-bound cells were recovered by positive selection following incubation with biotinylated anti-rat κ (clone MRK-1; BD Biosciences), and magnetic bead-labeled anti-biotin (Miltenyi Biotec). Both FDC-M1-positive and FDC-M1-negative cell fractions were recovered and lysed in HIV p24 ELISA lysis buffer (Beckman Coulter), followed by determination of HIV p24 levels in the lysates by ELISA (Beckman Coulter).

In vitro opsonization of HIV

For complement receptor-mediated opsonization, HIV MC99IIIBΔTat-Rev diluted to 50 ng/ml p24 was incubated with a 1/10 dilution of serum obtained from C57BL/6 mice for 60 min at 37°C. Serum that was heat-inactivated for 45 min at 56°C was included as negative control. The opsonized virus was then incubated with 2×10^5 mouse CD21-positive or CD21-negative K562 cells (25) in a 1:1 volume of virus to cells for 30 min at 4°C. For FcR-mediated opsonization, HIV MC99IIIBΔTat-Rev diluted to 50 ng/ml p24 was incubated with anti-HIV gp120 mAb 902 or mouse control IgG for 30 min at 37°C. The opsonized virus was then incubated with 2×10^5 human FcRIIb-positive or FcRIIb-negative L cells obtained from American Type Culture Collection (26), in a 1:1 volume of virus to cells for 30 min at 4°C. After extensive washing, the cells were lysed in HIV p24 ELISA lysis buffer (Beckman Coulter), followed by determination of HIV p24 levels in the lysates by ELISA (Beckman Coulter).

HIV RNA RT-PCR

Total RNA was extracted from single-cell LN suspensions using TRIzol (Invitrogen Life Technologies) according to the manufacturer's specifications. Levels of HIV-1 unspliced RNA were measured by RT-PCR as described (27). Briefly, RT-PCR was performed on triplicate samples of 500 ng of RNA using a TaqMan EZ-PCR kit (Applied Biosystems), according to the manufacturer's specifications. Standard curves were generated using in vitro transcribed HIV-1 RNA.

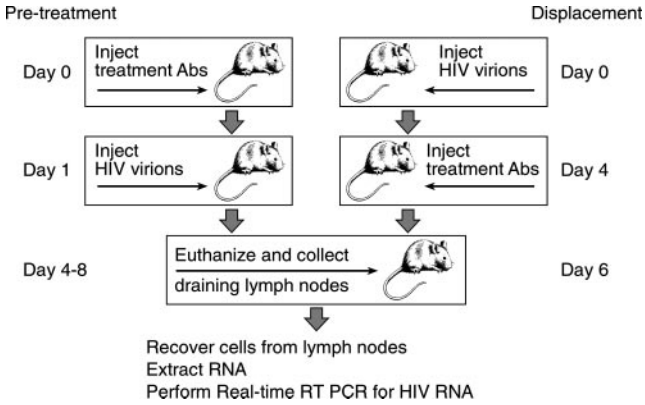

FIGURE 1. Scheme of study protocol. Mice were either injected with anti-HIV or anti-CD21 mAbs, followed by inoculation with HIV particles (pretreatment) (*left*) or inoculated with HIV particles, followed by injection of anti-CD21 mAb (displacement) (*right*). At day 4–8 postinfection, mice were euthanized, LN were harvested, single-cell suspensions were prepared by mechanical disruption, and RNA was isolated for measurement of HIV RNA by real-time RT-PCR.

Flow cytometry

Single-cell LN suspensions were stained for surface markers with the following Abs: FITC-labeled anti-CD4 mAb L3T4 and PE-labeled anti-CD21 mAb 7G6 were obtained from BD Biosciences; biotinylated anti-CD21 mAb 7E9 was obtained from S. Boackle (University of Colorado Health Sciences Center, Denver, CO); and PE-streptavidin was obtained from R&D Systems. The HIV envelope binding capacity of anti-HIV envelope mAbs Chessie 8, 178.1, and 902 were tested on CEM cells chronically infected with HIV-IIIB or CEM-NKR cells with goat anti-mouse IgG-PE serving as secondary Ab. For intracellular stains, cells were fixed (FACS lysing buffer; BD Biosciences) and permeabilized (Permeabilizing Solution 2; BD Biosciences) before the addition of Abs. Data were acquired on a FACSCalibur flow cytometer (BD Biosciences) and analyzed using FlowJo software (Tree Star).

Results

The strategies used to investigate the role of CD21 in trapping HIV virions to cells of mouse LN are illustrated in Fig. 1. A mouse model was chosen for the purpose of eliminating variables associated with productive HIV replication, and in doing so, enabling a better focus on mechanisms of extracellular virion trapping. To determine whether the presence of anti-HIV Abs was required for virion trapping, C57BL/6 mice were injected with or without anti-HIV Abs before injection of HIV (Fig. 1, *left*). To establish kinetics of HIV trapping, mice were euthanized and LN collected at 3, 5, and 7 days postinfusion. As shown in Fig. 2*A*, HIV was detected in the draining LN as early as 3 days postinfusion and appeared to plateau thereafter. Somewhat unexpectedly, there was no significant difference in HIV trapping to LN cells in the absence or presence of the anti-HIV gp41 mAb Chessie 8.

The choice of Chessie 8 was based on a model previously established to investigate HIV binding to FDC (17); however, the need for pre-existing anti-HIV Abs in vivo was not addressed per se in that study. Furthermore, epitope mapping has suggested that Chessie 8 is likely to recognize an intracytoplasmic domain of gp41 (19), which would limit its ability to bind intact virions and form immune complexes. To determine whether Chessie 8 recognized an extracellular epitope, we compared the binding of Chessie 8 and the anti-HIV gp120 mAb 902 to chronically HIV-infected

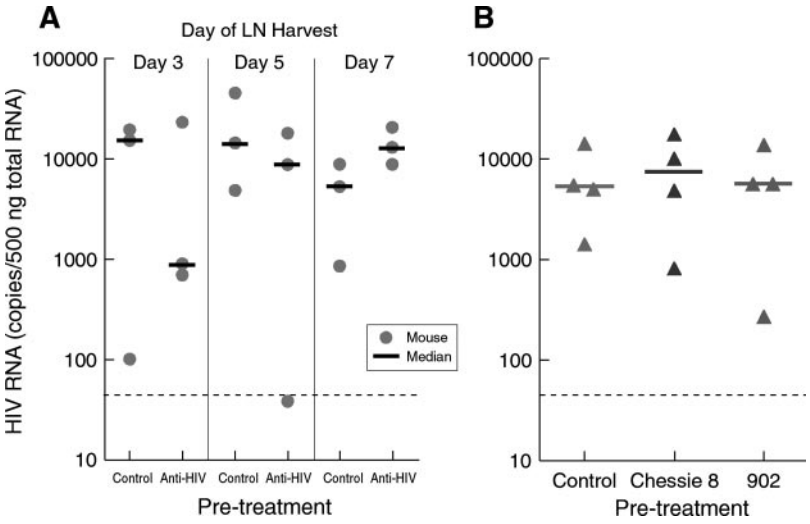

FIGURE 2. Evidence of HIV LN trapping in the absence of HIV-specific Abs. *A*, Kinetic comparison of HIV trapping by LN cells in the absence (control) or presence (anti-HIV) of anti-HIV gp41 mAb Chessie 8. Groups of C57BL/6 mice ($n = 3$) were inoculated per treatment and for each day of LN harvest, with the median measurement of the triplicate for each group indicated by a horizontal bar. *B*, Comparison of HIV trapping at day 5 in mice that had been pretreated with anti-HIV gp41 mAb Chessie 8, anti-HIV gp120 mAb 902, or control hybridoma supernatant. Groups of mice ($n = 4$) were inoculated per treatment. Median value is shown by horizontal bar. Amount of virus injected per mouse is $\sim 5 \times 10^9$ HIV RNA copies. Limit of detection is 38 copies of HIV RNA, depicted by dotted line.

CEM cells with or without cell permeabilization. As shown in Fig. 3, although the anti-HIV gp120 mAb 902 bound to HIV envelope expressed on the cell surface of chronically infected but not uninfected CEM cells, Chessie 8 did not bind either cell line. However, intracellular binding of Chessie 8 in the HIV-infected cells but not their uninfected counterpart was detected (Fig. 3), suggesting that although Chessie 8 recognizes an epitope of gp41, it would unlikely be exposed on the surface of intact virions. When the anti-HIV gp120 mAb 902 was injected into mice before infusion of HIV, it did not increase HIV trapping to LN cells when compared with mice that received control supernatant (Fig. 2*B*). Considering that both Chessie 8 and 902 are of the IgG1 isotype and that in mice IgG2 and IgG3 can activate the classical complement pathway more efficiently than IgG1 (28–30), we repeated the experiment with the IgG2a anti-HIV mAb 178.1, which recognizes an exposed epitope of HIV gp120 (21 and data not shown). Preinjection with mAb 178.1 also did not lead to any significant difference in HIV trapping compared with control or with mAb 902 (data not shown). Taken together, these data suggest that extracellular trapping of HIV to LN can occur in the absence of pre-existing IgG anti-HIV Abs, whether or not they recognize external epitopes of HIV envelope.

To determine more specifically which cells in the LN tissue were responsible for the extracellular trapping of HIV, mice were injected with HIV virions and at day 5 single-cell suspensions were prepared from LN tissue and fractionated into FDC-enriched and FDC-depleted populations. As shown in Fig. 4*A*, the FDC-specific Ab FDC-M1 recognized 48% of FDC-enriched LN cells, compared with background levels in the FDC-depleted fractionated and unfractionated LN cells. This relatively low level of enrichment reflects a balance between difficulties in purifying intact FDC (24) and the loss of HIV virions with prolonged enzymatic digestion (data not shown). Nonetheless, the 48% FDC enrichment was associated with a 64-fold increase in levels of HIV detected in the FDC-enriched population compared with the FDC-depleted population (Fig. 4*B*). These data are consistent with studies demonstrating that FDC account for the major extracellular reservoir in the lymphoid tissues of HIV-infected individuals (14) and of mice injected with HIV virions (17). However, cells that remained attached to the FDC may also have contributed to viral burden,

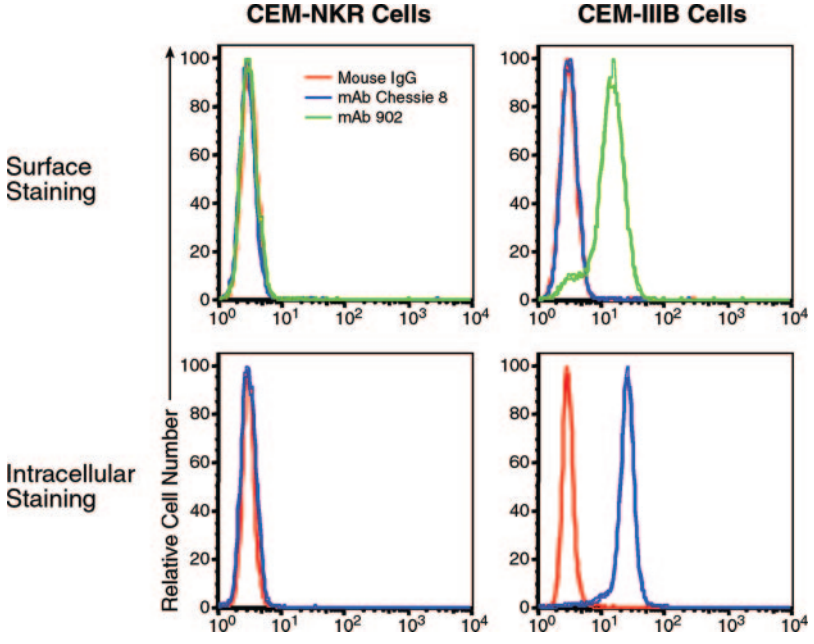

FIGURE 3. Binding of anti-HIV gp41 mAb Chessie 8 and anti-HIV gp120 mAb 902 to CEM cells chronically infected with HIV strain IIIB (CEM-IIIB) and control (CEM-NKR) cells. Cells were stained without (*top panels*) or with (*bottom panels*) permeabilization. Negative Ab control consisted of mouse IgG.

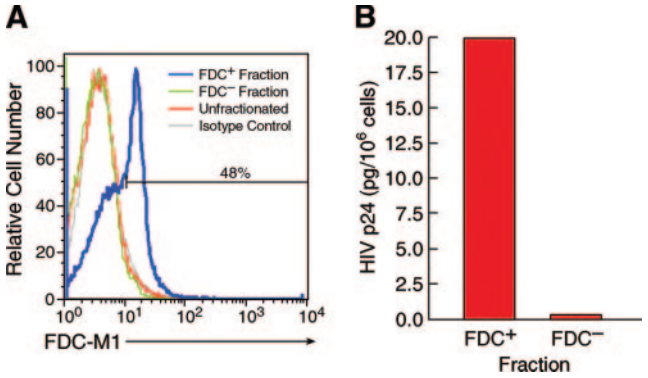

FIGURE 4. FDC are responsible for majority of HIV LN trapping. *A*, LN cells were enriched in FDC by fractionation with anti-FDC mAb FDC-M1. Each fraction and unfractionated LN cells were stained with FDC-M1 or isotype control. *B*, HIV p24 ELISA was performed on cell lysates from FDC-enriched (FDC^+) and FDC-depleted (FDC^-) fractions. Virus injected per mouse was 600 ng of HIV p24.

including B cells that have been shown to trap HIV (15) and to form tight clusters with FDC (7).

FcR have been shown to mediate trapping of immune-complexed HIV by FDC in vitro (31), a finding we confirmed with L cells expressing FcRIIb (Fig. 5*A*), which is the major FcR expressed on FDC (32). However, several studies have suggested that in vivo, CD21 may play a more prominent role than FcR in the trapping of HIV by FDC and B cells of LN (14, 15, 18, 33). Similar to anti-human CD21 Abs, several anti-mouse CD21 mAbs, including 7G6, have both C3 ligand-blocking and CD3 ligand-displacing potential (34). Both of these activities were considered in the context of HIV binding to mice LN cells in vivo.

To verify that the murine model described in Fig. 1 was appropriate for studies of the role of complement receptors in HIV trapping to LN cells, the HIV virions used for injection into C57BL/6 mice were treated in vitro with serum obtained from C57BL/6 mice and incubated with CD21-positive or CD21-negative K562 erythroleukemia cells (25). As shown in Fig. 5*B*, CD21-positive K562 cells captured HIV particles, whereas no virus was captured by CD21-negative K562 cells or under conditions in which the serum was heat-inactivated. These data demonstrate that HIV particles can be opsonized with CD21-binding complement fragments derived from the serum of C57BL/6 mice.

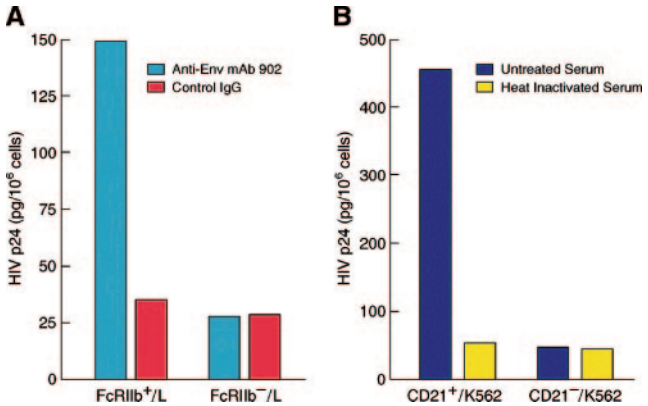

FIGURE 5. In vitro opsonization of HIV virions. *A*, HIV virions opsonized with anti-HIV gp120 mAb 902 or control IgG were incubated with FcRIIb-positive ($FcRIIb^+$) or FcRIIb-negative ($FcRIIb^-$) L cells. *B*, HIV virions opsonized with complement fragments from C57BL/6 mouse serum with or without heat-inactivation were incubated with CD21-positive ($CD21^+$) or CD21-negative ($CD21^-$) K562 cells. HIV p24 ELISA was performed on cell lysates.

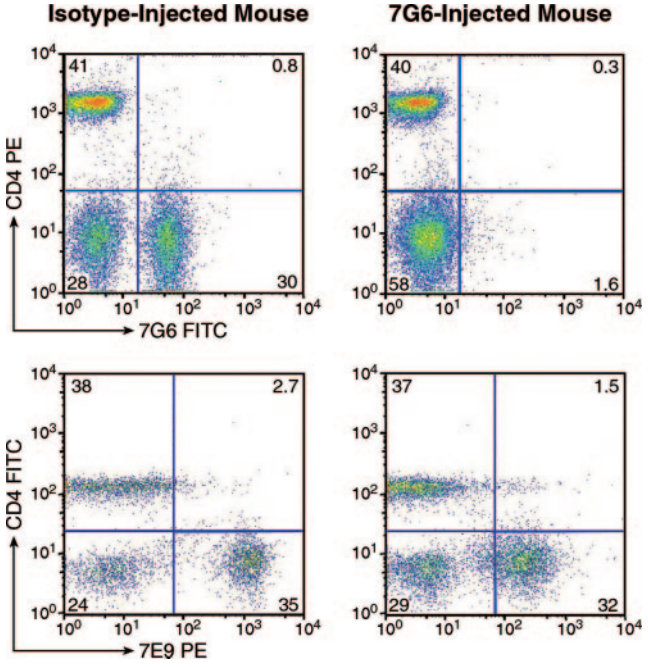

FIGURE 6. Surface marker expression patterns of LN cells isolated from mice treated with anti-CD21 mAb 7G6 (*right panels*) or isotype control mAb (*left panels*), and stained with anti-CD4 mAb and anti-CD21 mAb 7G6 (*top panels*) or with 7E9 (*bottom panels*). A pattern of staining following treatments described in Fig. 7*A* is shown.

When wild-type C57/BL6 mice were injected with mAb 7G6, a substantial proportion of CD21-expressing LN cells bound the mAb, as demonstrated by a 95% reduction of FITC-conjugated 7G6 binding when the LN cells were analyzed ex vivo, and compared with a negligible loss in percentage of binding by the non-competing anti-CD21 mAb 7E9 (Fig. 6). However, and consistent with previous findings (34), the decreased intensity of mAb 7E9 binding in mAb 7G6-treated compared with isotype control-treated mice (Fig. 6) indicated that injection of mAb 7G6 led to a down-regulation or internalization of CD21. Although not tested in this study, previous data also indicate that the effect of mAb 7G6 on CD21 expression is transient, lasting ~7 days (34). In addition, again consistent with our data (Fig. 6), treatment with mAb 7G6 does not lead to depletion of B cells or FDC target cells (34).

To directly evaluate the role of CD21 in the extracellular trapping of HIV virions to LN cells, mice were injected with the anti-mouse CD21 mAb 7G6 1 day before injection of HIV (Fig. 1, *left*). The preinjection of mAb 7G6 led to a significant reduction in HIV trapping to LN cells, in the order of an >90% reduction compared with mice treated with the isotype control mAb (Fig. 7*A*). To further demonstrate a role for CD21 in HIV trapping to LN cells, HIV virion-binding capacity was compared in CD21-deficient ($Cr2^{-/-}$) mice and C57BL/6 wild-type mice from which they were derived. In four separate experiments, no HIV could be measured in the LN recovered from $Cr2^{-/-}$ mice, whereas HIV was consistently observed in LN cells of the wild-type mice (Fig. 7*B*), confirming a role for CD21 in HIV virion trapping by LN cells.

Previous studies have also suggested a potential role for anti-CD21 mAbs in displacing HIV virions that are already bound to LN cells (14, 15, 18), mimicking conditions that would be most frequently encountered in HIV-infected patients. Such a role for mAb 7G6 was tested by performing the injections in reverse: HIV

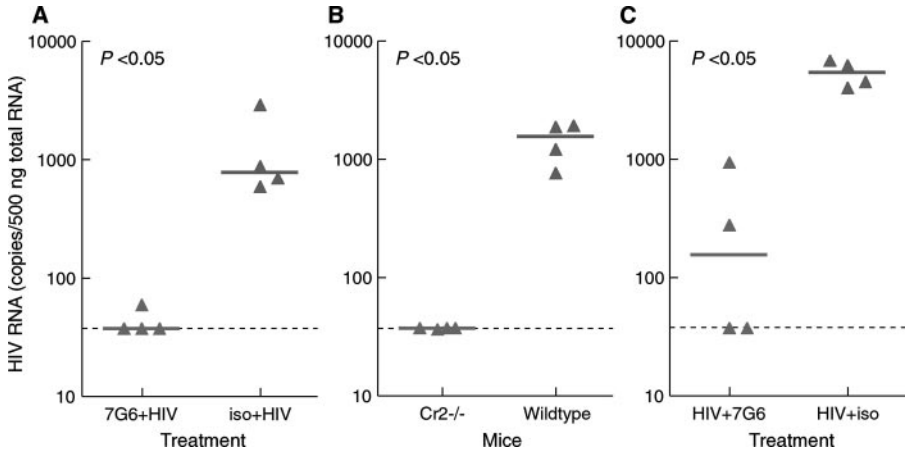

FIGURE 7. Essential role for CD21 in LN trapping of HIV. *A*, Blocking experiments were performed by pretreating mice with anti-CD21 mAb 7G6 (7G6+HIV) or isotype control mAb (iso+HIV) followed by inoculation. *B*, Comparison of HIV LN trapping in $Cr2^{-/-}$ and wild-type mice. *C*, Displacement experiments were performed by inoculating mice with HIV followed by treatment with anti-CD21 mAb 7G6 (HIV+7G6) or isotype control mAb (HIV+iso). Groups of mice ($n = 4$) were inoculated per treatment, with median values shown by horizontal bars. Data shown are representative of three to four independent experiments. Virus injected per mouse is $\sim 5 \times 10^9$ HIV RNA copies. Limit of detection is 38 copies of HIV RNA, depicted by dotted line.

virions were injected first, followed by an injection of mAb 7G6 or isotype control at day 5, and collection of LN 2 days later (Fig. 1, *right*). The infusion of mAb 7G6 led to a 40- to 50-fold reduction in LN-bound HIV compared with mice treated with isotype control mAb (Fig. 7*C*). Taken together, these finding indicate that the anti-CD21 mAb 7G6 not only blocks binding of HIV virions to LN cells but also displaces virus that is already bound.

Discussion

We have used a noninfectious mouse model to gain new insight into the mechanisms associated with HIV extracellular trapping in lymphoid tissues. Within the confines of the model studied, the present data indicate that CD21 is the major HIV virion-trapping receptor in LN tissues, that FDC are likely to be responsible for the majority of extracellular HIV trapping in LN tissues, and that anti-CD21 mAbs can reduce the amount of virus associated with lymphoid tissue by displacing bound virus. We also find evidence that trapping of HIV virions to LN cells can occur in the absence of highly specific anti-HIV Abs. In this regard, the requirement for neutralizing Abs against HIV for efficient trapping of HIV by FDC has not been fully addressed. The few in situ studies conducted on a cross-section of patients from acute to later stages of HIV infection offer a mixed picture in this regard, and by all accounts the paucity of patients in each category was seen as a major reason for the apparent discrepancies. One earlier study (35) reported delayed detection of HIV in FDC networks, whereas in another study, large amounts of FDC-associated HIV were observed before the appearance of neutralizing Abs (36). In the experimental SIV model, there is evidence for very early establishment of a high viral burden in lymphoid tissues (36–38), and evidence of FDC-associated SIV or simian HIV has been documented early after infection, possibly before the appearance of virus-specific IgG Abs (39, 40). Consistent with these observations, complement-dependent binding of HIV to FDC in vitro has been demonstrated to occur in the absence of HIV-specific Abs (41). More recently, components of the innate immune system involving lectins have also been proposed for both complement-dependent opsonization of HIV (42) and complement-independent binding of HIV to FDC (43). However, the in vivo significance of these proposed pathways of HIV trapping remains to be determined.

The use of mouse models to study extracellular trapping of HIV provides numerous advantages, including the potential for genetic manipulation and a more biologically relevant system when compared with most in vitro approaches. There are also limitations, including complement-related differences between mice and humans and unintended effects of using human virus in a mouse model. One of the more important differences between mouse and human complement receptors is that CD35 in mice is a splice variant of CD21, whereas it is a separate gene in humans and has a broader expression and functional profile (44). Nonetheless, the role of these two complement receptors relative to immune complex binding are thought to be sufficiently similar for mice models to provide important insight into human disease (45, 46). A second limitation relates to the possibility of enhanced complement activation and complement-mediated lysis resulting from the injection of human cell-derived HIV virions into mice (47). However, human components in the HIV virions are unlikely to be the sole activating source of complement given the various mechanisms by which Ag, and HIV in particular, have been shown to induce complement activation in the absence of Ag-specific Abs (42, 43, 48, 49). With regard to enhanced virolysis, this phenomenon may explain why relatively low levels of virus were recovered from a high input of virions, and why complement-activating anti-HIV Abs did not lead to increased HIV trapping. A balance between complement-mediated trapping and virolysis has been proposed in HIV disease (50), and would be consistent with our present data and previous studies in the mouse model indicating that a stable level of virus trapping is maintained (17). Finally, as the factors required for HIV propagation in mouse cells become elucidated, it may be possible in the future to address more directly the cross-species effects as described.

Two mechanisms of Ag trapping on FDC have been described in vivo, one involving complement receptors that have been shown to play an essential role in primary immune responses (51), and the other involving FcR that have been shown to be more important in regulating recall responses (32). Our present and past data (15) would be more consistent with the former mechanism and in agreement with studies on lymphoid tissues isolated from HIV-infected individuals, which show CD21-dependent trapping of infectious HIV (33) and a near complete elimination of the HIV signal following incubation of the tissue sections with an anti-CD21 mAb (14). However, there are indications that under certain conditions, perhaps in the presence of high levels of HIV-specific IgG Abs and well-developed germinal centers, FcR may play a

more prominent role. This potential has been demonstrated with FDC in vitro (31), a finding we confirmed with FcRIIb-expressing cells. However, we did not test anti-HIV mAb infusion in the context of blocking or displacing with anti-CD21 or anti-FcRIIb mAbs. In the latter case, this testing would likely require a different strategy as the strategy illustrated in Fig. 1 induces few germinal center reactions (our unpublished observations) and FDC express high levels of FcRIIb only after germinal centers are formed (32).

We and others report that anti-human CD21 mAbs not only block but also displace HIV virions ex vivo from B cells and from follicular areas of LN tissue sections (14, 15, 18). Considering that B cells and FDC both express CD21 and similarly bind immune complexes through C3 components, there is good reason to believe that the HIV binding mechanisms to B cells and FDC are similar. In the present murine study, we provide in vivo evidence that anti-mouse CD21 mAbs possess virion-displacing activities, and hence this class of Abs has the potential for reducing viral burdens. The active displacement of virions from FDC may accelerate the decay of a relatively hardy extracellular reservoir, as evidenced from in vivo and in vitro models (3, 17) and due to the high valency of receptor-virus interactions estimated for FDC (52). Furthermore, given the insensitivity of the FDC reservoir to current antiretroviral therapy this avenue also represents a different and possibly additive approach for reducing and controlling viral replication.

In summary, our findings provide new insight into the mechanisms associated with extracellular trapping of HIV in lymphoid tissues and the possibility of targeting this HIV reservoir to further reduce the viral burden in infected patients. The findings of the present study, combined with several previous in vitro studies on B cells and LN tissues of human subjects, offer strong support to the notion that CD21 plays a major role in the extracellular trapping of HIV in lymphoid tissues, which represent a major reservoir for HIV. Anti-CD21 mAbs with strong C3 ligand-displacing attributes have been identified for both human and mouse targets and the anti-human CD21 mAbs have also been shown to recognize simian CD21 (data not shown) and are currently being tested in an experimental SIV model. Finally, the use of anti-CD21 mAbs to reduce the HIV-FDC reservoir in HIV-infected patients may not only accelerate viral decay but also help restore the FDC network and the important role an intact FDC network plays in the induction of an efficient immune response (6, 7).

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Disclosures

The authors have no financial conflict of interest.

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