Alterations in Cholesterol Metabolism Restrict HIV-1 *Trans* Infection in Nonprogressors

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ABSTRACT HIV-1-infected nonprogressors (NP) inhibit disease progression for years without antiretroviral therapy. Defining the mechanisms for this resistance to disease progression could be important in determining strategies for controlling HIV-1 infection. Here we show that two types of professional antigen-presenting cells (APC), i.e., dendritic cells (DC) and B lymphocytes, from NP lacked the ability to mediate HIV-1 *trans* infection of CD4$^+$ T cells. In contrast, APC from HIV-1-infected progressors (PR) and HIV-1-seronegative donors (SN) were highly effective in mediating HIV-1 *trans* infection of T cells with HIV-1 was comparably efficient among NP, PR, and SN. Lack of HIV-1 *trans* infection in NP was linked to lower cholesterol levels and an increase in the levels of the reverse cholesterol transporter ABCA1 (ATP-binding cassette transporter A1) in APC but not in T cells. Moreover, *trans* infection mediated by APC from NP could be restored by reconstitution of cholesterol and by inhibiting ABCA1 by mRNA interference. Importantly, this appears to be an inherited trait, as it was evident in APC obtained from NP prior to their primary HIV-1 infection. The present study demonstrates a new mechanism wherein enhanced lipid metabolism in APC results in remarkable control of HIV-1 *trans* infection that directly relates to lack of HIV-1 disease progression.

IMPORTANCE HIV-1 can be captured by antigen-presenting cells (APC) such as dendritic cells and transferred to CD4 helper T cells, which results in greatly enhanced viral replication by a mechanism termed *trans* infection. A small percentage of HIV-1-infected persons are able to control disease progression for many years without antiretroviral therapy. In our study, we linked this lack of disease progression to a profound inability of APC from these individuals to *trans* infect T cells. This effect was due to altered lipid metabolism in their APC, which appears to be an inherited trait. These results provide a basis for therapeutic interventions to control of HIV-1 infection through modulation of cholesterol metabolism.

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Most human immunodeficiency virus type 1 (HIV-1)-infected individuals display ongoing viral replication concomitant with progressive CD4$^+$ T cell depletion which, when left untreated with combination antiretroviral therapy (ART), leads to the development of AIDS. It has been recognized, however, that a small percentage of chronically infected individuals are able to control disease progression for many years in the absence of ART. They have been classified as HIV-1 controllers on the basis of virologic criteria, including elite controllers (<1% of HIV-1-infected individuals) with persistently undetectable numbers of HIV-1 RNA copies/ml of plasma (1) and viremic controllers with plasma HIV-1 RNA levels of 50 to 2,000 copies/ml (2). A third group of nonprogressors (NP; 2 to 15% of HIV-1-infected individuals) exhibit persistently high to moderate numbers of CD4$^+$ T cells with variable viral loads (3). Understanding the virologic and immunologic mechanisms of disease control in these individuals could be important in developing therapeutics and vaccines for controlling and preventing HIV-1 infection.

HIV-1 infection undergoes enhanced replication in CD4$^+$ T cells after transmission through dendritic cells (DC) (4), monocytes/macrophages (5), and B lymphocytes (6), termed *trans* infection (7). Such *trans* infection mediated *in vitro* by these professional antigen-presenting cells (APC) is significantly greater than direct *cis* infection of either APC or T cells. Given the intimate association of APC and T cells in lymphoid tissue, HIV-1 *trans* infection could be important in disease progression (8).

Cholesterol is essential for HIV-1 *trans* infection mediated by DC and macrophages (9, 10). Moreover, treatment of DC with the nuclear receptor (NR) ligands peroxisome proliferator-activated receptor γ (PPARγ) and liver X receptor (LXR) inhibits HIV-1 *trans* infection by increasing cholesterol efflux through modulation of ATP-binding cassette transporter A1 (ABCA1) activity (9). Enhanced NR expression of the cholesterol transporter ABCA1 also reduces HIV-1 *cis* replication in macrophages and T cells (11, 12). Thus, we postulated that enhanced cholesterol metabolism could be related to the efficiency of HIV-1 *trans* infection in NP.

We therefore investigated whether HIV-1 *trans* infection mediated by APC from NP is less efficient than in HIV-1-infected

TABLE 1 Immunologic, virologic, and genotypic characteristics of NP and PR

Subject or parameter	Infection duration (yr)[a]	Mean no. of CD4$^+$ T cells $\pm$ SE[b]	Mean HIV-1 load $\pm$ SE[c]	HLA class I genotype	CCR5Δ32[d]
NP1	>10	1,007 $\pm$ 31	571 $\pm$ 90	15:01/42:01	WT/WT
NP2	>25	1,259 $\pm$ 62	4,764 $\pm$ 4,432	14:02/47:01	WT/WT
NP3	>8	924 $\pm$ 62	3,055 $\pm$ 566	39:10/53:01	WT/WT
NP4	>8	743 $\pm$ 59	3,242 $\pm$ 1,931	35:01/81:01	WT/WT
NP5	>8	622 $\pm$ 29	67,006 $\pm$ 26,528	35:01/41:02	WT/WT
NP6	>12	541 $\pm$ 31	1,014 $\pm$ 132	15:10/42:01	WT/WT
NP7	19.5	759 $\pm$ 57	20,893 $\pm$ 7,585	15:01/44:02	WT/WT
NP8	16.5	783 $\pm$ 27	50,105 $\pm$ 23,968	18:01/51:01	WT/Δ32
Mean $\pm$ SE[e]		878 $\pm$ 30	15,871 $\pm$ 3,980		
PR1	6.5	204 $\pm$ 30	429,049 $\pm$ 91,578	08:01/35:03	WT/WT
PR2	8.5	443 $\pm$ 28	59,065 $\pm$ 12,835	40:01/44:02	WT/WT
PR3	2.9	308 $\pm$ 51	63,159 $\pm$ 36,958	37:01/06:02	WT/WT
PR4[f]	>5	350 $\pm$ 57	147,660 $\pm$ 65,974	38:01/49:01	WT/WT
PR5[f]	2.1	232 $\pm$ 49	396,572 $\pm$ 136,552	50:01/57:01	WT/WT
PR6[f]	3.8	245 $\pm$ 125	188,576 $\pm$ 45,187	08:01/51:01	WT/WT
PR7	5.2	362 $\pm$ 65	40,604 $\pm$ 12,234	13:02/35:01	WT/WT
PR8	6.1	374 $\pm$ 28	117,151 $\pm$ 28,059	08:01/35:01	WT/WT
Mean $\pm$ SE[e]		391 $\pm$ 21	212,956 $\pm$ 32,621		

[a] Years of HIV-1 infection prior to ART, death, or discontinuation in the MACS.
[b] Number of cells/mm³.
[c] Number of HIV-1 RNA copies/ml of plasma.
[d] WT, wild type.
[e] Mean $\pm$ SE CD4$^+$ T cell counts and viral loads were determined at approximately 6-month intervals over the indicated numbers of years of infection.
[f] Deceased.

progressors (PR) and hence underlies their lack of disease progression. For this we examined the relationship of two types of APC, i.e., myeloid DC and B cells, with cholesterol levels and ABCA1 activity in HIV-1 *trans* infection and disease progression in NP in the Multicenter AIDS Cohort Study (MACS), a longitudinal study of HIV-1 infection in men who have sex with men (13). These NP had persistently stable CD4$^+$ T cell counts (>500 cells/mm³) and low viremia over many years of chronic HIV-1 infection. We show that DC and B cells from NP were unable to *trans* infect CD4$^+$ T cells with HIV-1, in contrast to the efficient *trans* infection mediated by these APC from PR and uninfected, HIV-1-seronegative donors (SN). CD4$^+$ T cells from all three groups supported similar levels of direct *cis* infection. Lack of HIV-1 *trans* infection was related to lower cholesterol contents and higher reverse cholesterol transporter ABCA1 levels in DC and B cells but not T cells from NP than in those from PR and SN. These observations advance our understanding of HIV-1 disease progression and provide new, important clues for control of HIV-1 infection.

RESULTS

Immunologic, virologic, and genotypic characteristics of NP and PR. NP ($n = 8$) had documented infection with HIV-1 in the MACS for a median of 11 years (range, >8 to 19.5 years) while consistently maintaining absolute CD4$^+$ T cell counts of >500/mm³ (mean $\pm$ standard error [SE], 878 $\pm$ 30/mm³) with a slope of 6.5 (95% confidence interval, 0.19 to 12.96) (Table 1). We compared these subjects to PR ($n = 8$) who consistently had CD4$^+$ T cell counts of <500/mm³ (mean $\pm$ SE, 391 $\pm$ 21/mm³; $P < 0.001$ compared to NP) with a slope of -6.8 (95% confidence interval, -11.86 to -1.74; $P < 0.001$ compared to NP) over a median of 5.1 years of infection (range, 2.1 to 8.5 years). NP had a significantly lower viremia level than PR over the course of HIV-1 infec-

tion (mean $\pm$ SE numbers of HIV-1 RNA copies/ml, 15,847 $\pm$ 3,980 and 212,965 $\pm$ 32,621, respectively; $P = 0.0001$) (Table 1). The NP and PR were ART naive and not undergoing treatment with cholesterol-lowering drugs throughout the course of HIV-1 infection. The NP and PR did not have a predominance of known major protective genotypes, as no NP and two PR carried the HLA B*5701 allele (14) and one NP and no PR were heterozygous for the CCR5Δ32 mutation (15) (Table 1).

DC and B cells from NP do not mediate HIV-1 *trans* infection of CD4$^+$ T cells. DC or B cells from the NP and PR were loaded with HIV-1 BaL (CCR5 coreceptor [R5] tropic) at 3 pg of p24/10^6 cells and then mixed with autologous CD4$^+$ T cells as described in Materials and Methods. We chose this low input virus multiplicity of infection (MOI) because it does not result in efficient *cis* infection of CD4$^+$ T cells yet is highly effective in APC-to-T-cell *trans* infection (6). Strikingly, neither DC nor B cells from the eight NP were able to *trans* infect T cells, in contrast to the efficient *trans* infection mediated by DC (Fig. 1A) and B cells (Fig. 1B) from the eight PR and seven SN ($P = 0.03$, two-way analysis of variance [ANOVA]). Levels of p24 were negative (i.e., below the limit of detection of the p24 enzyme-linked immunosorbent assay [ELISA]) throughout the 16 days of APC–T-cell coculture for seven of the eight NP. In the other NP, there was a low level (110 pg/ml) of p24 detected at 16 days in the B-cell–T-cell cocultures. Notably, to ensure that our *trans* infection system was operational, in every NP *trans* infection experiment we included parallel cocultures of cryopreserved DC and B cells with autologous T cells from an SN whose APC we had previously shown mediated HIV-1 *trans* infection. These control cultures expressed levels of p24 that increased from a mean $\pm$ SE of 537 $\pm$ 43 pg/ml on day 4 to 15,046 $\pm$ 4,179 pg/ml on day 16 (data not shown). Moreover, completely impaired HIV-1 *trans* infection was noted when using
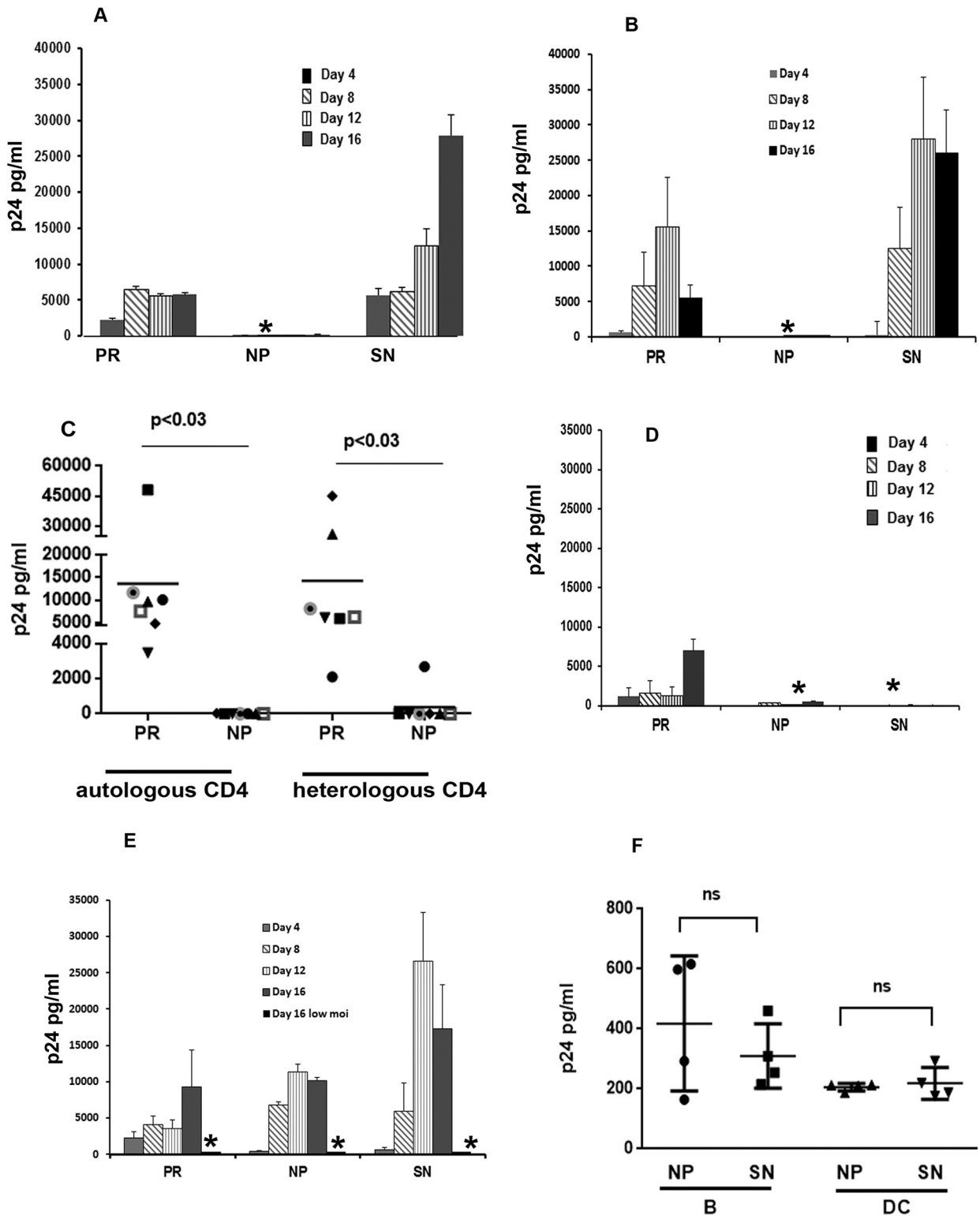
FIG 1 DC and B cells from NP do not mediate HIV-1 *trans* infection of autologous or heterologous CD4+ T cells. (A to C) APC derived from eight PR, eight NP, and seven SN were loaded with R5 HIV-1 BaL and cocultured with autologous (A and B) or heterologous (C; *n* = 7 per group) CD4+ T cells. Concentrations of HIV-1 p24 in the cell culture supernatants were tested every 4 days for 16 days. Individuals were independently tested multiple times with similar results (see the text). *P* < 0.03 by two-way ANOVA. (D) CD4+ T cells from the subjects tested in the experiments shown in panels A and B were cultured for the indicated times to test endogenous autologous virus production. (E) CD4+ T cells from the same subjects as in panel A were infected in *cis* with 3 or 300 pg/ml of HIV-1 BaL, and p24 concentrations were determined as described above. For clarity, p24 concentrations in cultures loaded with the lower MOI are shown only at the latest time point (day 16). Data represent mean p24 concentrations from triplicate wells ± SE. (F) B cells or DC from four NP (NP1, NP2, NP3, and NP4) and four SN were incubated with HIV-1, and virus capture was measured by p24 ELISA. *, below the limit of detection; ns, no statistically significant difference.
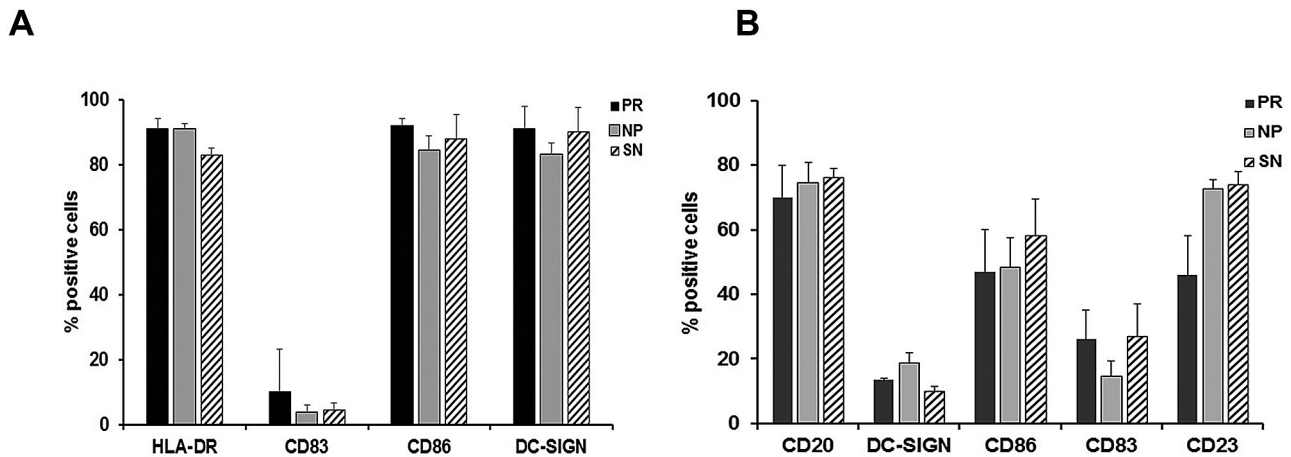
A

B

FIG 2 DC and B cells from NP do not have altered surface marker expression. DC (A) and B cells (B) from seven PR, eight NP, and seven SN were stained with MAbs against the indicated cell markers. Bars represent the mean percentages of positive cells ± the SE.

APC and T cells obtained at two to four additional time points over 36 months from seven of the eight NP who were available for retesting (data not shown).

We next compared *trans* infection of autologous to heterologous $CD4^+$ T cells derived from an SN by APC from seven PR and seven NP that were loaded with HIV-1 *in vitro*. The results show that B cells from PR were capable of mediating similar high levels of *trans* infection of HIV-1 by 12 days of both autologous and heterologous $CD4^+$ T cells (Fig. 1C), while B cells from NP were not capable of *trans* infecting either T cell target, with the exception of one NP, where we detected a small amount of HIV-1 p24 at 16 days in cocultures of B cells and heterologous $CD4^+$ T cells. Similar results were obtained with DC and T cells derived from three NP (data not shown). These observations raised the question of whether HIV-1 detected in APC–T-cell cocultures from PR was derived from endogenous virus harbored in the autologous $CD4^+$ T cells and therefore did not constitute true *trans* infection of input exogenous virus. To address this, activated $CD4^+$ T cells derived from PR, NP, and SN were cultured without the addition of exogenous HIV-1 and tested for production of p24 over 16 days. The data indicate that only T cells from PR produced endogenous HIV-1, with a peak level of 7,014 pg of p24 at 16 days (Fig. 1D).

We next addressed the possibility that $CD4^+$ T cells from NP were nonpermissive for virus replication. We found that activated, autologous $CD4^+$ T cells from all three study groups could be directly infected with a high MOI of HIV-1 (300 pg/10^6 cells) and produced comparable amounts of virus by 12 to 16 days (no statistically significant difference) (Fig. 1E). In sharp contrast, there was no evidence of productive *cis* infection of $CD4^+$ T cells in the three study groups with the same small amount of HIV-1 (3 pg/10^6 cells) as in the *trans* infection cocultures (16 days low MOI, Fig. 1E). B cells and DC from NP also bound HIV-1 with the same efficiency as those from SN (Fig. 1F), excluding the possibility that the reduced *trans* infection observed in NP was due to poor virus binding to APC. Finally, we determined if the observed impaired ability to transfer HIV-1 was due to an altered phenotype in the APC from NP compared to those from PR or SN. As shown in Fig. 2A and B, there was no difference in the expression of several cellular markers in either DC or B cells from the three groups of

study subjects. In particular, we found no difference among the study groups in the expression on DC or B cells of DC-SIGN (CD209), a C-type lectin that is centrally involved in DC- and B-cell-mediated *trans* infection (6).

B cells obtained from NP before primary HIV-1 infection do not mediate HIV-1 *trans* infection of $CD4^+$ T cells. Our results indicated that APC derived from NP at various times after HIV-1 infection are uniquely incapable of mediating HIV-1 *trans* infection. We next determined whether the inability of APC from NP to mediate HIV-1 *trans* infection was acquired after HIV-1 infection by comparing B cells from two PR and two NP obtained approximately 1 to 4 years prior to documented HIV-1 seroconversion in the MACS. We did not have sufficient cryopreserved peripheral blood mononuclear cells (PBMC) to test DC. The HIV-1 antibody-negative NP and PR were confirmed to be virus negative by lack of detection of HIV-1 RNA in their blood plasma samples obtained before seroconversion (data not shown). We found that B cells derived from the two PR both before and after seroconversion were able to *trans* infect $CD4^+$ T cells with comparable efficiency (Fig. 3A). Levels of p24 in the B and T cell cultures were higher when T cells obtained before rather than after seroconversion were used. In contrast, B cells derived before and after HIV-1 seroconversion from both NP did not *trans* infect their $CD4^+$ T cells (Fig. 3A). Similar to the NP, levels of HIV-1 were higher when B cells were cocultured with T cells obtained before rather than after seroconversion. However, autologous $CD4^+$ T cells from PR and NP were similarly susceptible to direct *cis* infection with HIV-1 at time points both before and after HIV-1 infection (Fig. 3B).

The results indicate that the biological property of lack of *trans* infection in NP was present in their circulating-blood B cells before these subjects were infected with HIV-1. Therefore, the inability to mediate *trans* infection is not a result of direct or indirect conditioning of APC by HIV-1 or other cofactors acquired after HIV-1 seroconversion and could be an inherited trait.

Inhibition of cholesterol metabolism impairs B-cell-mediated HIV-1 *trans* infection. Treatment of DC with the NR ligands PPARγ and LXR inhibits HIV-1 *trans* infection by increasing cholesterol efflux via alteration of ABCA1 activity (9). Of note is that PPARγ is expressed by B cells (16). Moreover, B cells ex-
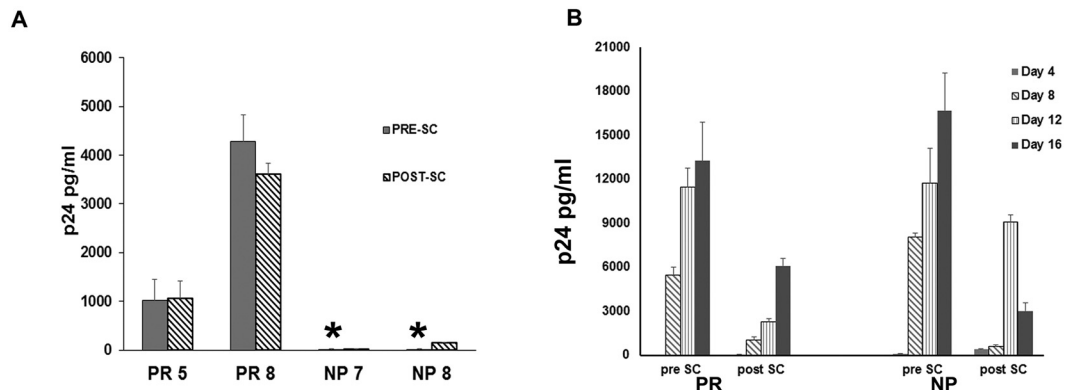
FIG 3 Lack of *trans* infection is not a consequence of HIV-1 infection. (A) B cells from two NP (NP7, NP8) and two PR (PR5, PR8) collected before (gray bars) and after (diagonal bars) HIV-1 seroconversion (SC) were loaded with HIV-1 and cocultured with autologous CD4$^+$ T cells. Supernatants were tested after 12 days of coculture, and p24 levels were measured by ELISA. (B) Autologous CD4$^+$ T cells from subjects used in Fig. 2A were loaded with HIV-1 BaL at an MOI of 300 pg of p24/10^6 cells and cultured in parallel to assess susceptibility to *cis* infection. *, below the limit of detection.

press LXR, and its activation does not interfere with CD40 L- or interleukin-4 (IL-4)-mediated B cell activation and proliferation (17). Thus, to examine whether this mechanism could be involved in the lack of *trans* infection mediated by APC from NP, we first determined whether treatment of B cells with the PPAR-γ ligand ciglitazone and the LXR ligand TO-901317 interfered with the ability of these cells to *trans* infect T cells. When B cells from SN were treated with either ligand, we observed a 60 to 70% reduction in the levels of HIV-1 *trans* infection of T cells (Fig. 4A). Treatment with either ligand did not greatly alter cell viability, which was >75% for each treatment. This observation parallels that noted with DC-mediated *trans* infection (9) and indicates that alteration in cell cholesterol metabolism could affect the efficiency of viral *trans* infection by B cells.

We next examined the effect of inhibition of cholesterol metabolism in *trans* infection by treatment with 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors (statins), which have been shown to impede cholesterol synthesis and decrease viral loads in HIV-1-infected cells *in vitro* and in chronically HIV-1-infected patients (18). For this, we treated DC and B cells from SN with lovastatin prior to loading the cells with HIV-1 and measuring *trans* infection of autologous CD4$^+$ T cells. Cell viability after treatment was >80%. Treatment with this statin reduced the ability of DC and B cells to mediate *trans* infection by approximately 60 and 90%, respectively (Fig. 4B).

For a more direct measurement of the role of cholesterol in APC-mediated HIV-1 *trans* infection, we treated B cells from SN with the lipophilic cyclic oligosaccharide methyl-β-cyclodextrin (BCD) to remove membrane cholesterol prior to loading with HIV-1 and coculture with CD4$^+$ T cells. This treatment completely abrogated HIV-1 *trans* infection compared to untreated B cells (Fig. 4C). Treatment of B cells with TO901317 or BCD decreased the cellular cholesterol content by 75 and 50%, respectively (Fig. 4D). Cell viability after treatment was >75%.

Taken together, these findings indicate that impairment of cholesterol metabolism or depletion of cellular cholesterol content decreases the ability of both types of APC to *trans* infect CD4$^+$ T cells. These APC appear to have pathways related to cholesterol metabolism in this *trans* infection process in common.

Total cell cholesterol content is reduced in APC of NP. Since cholesterol metabolism and/or transport is important in *trans* in-

fection, we investigated whether DC and B cells from NP were deficient in cholesterol content. The data show that these APC from NP had the smallest amount of cholesterol compared to APC from SN and PR (Fig. 5A and B; $P < 0.05$). In contrast, the levels of cholesterol in CD4$^+$ T cells from the three groups were similar (Fig. 5C; no statistically significant difference). These results support the idea that the impaired ability of APC from NP to *trans* infect CD4$^+$ T cells is related to an altered cholesterol content of APC but not T cells.

Cholesterol reconstitution confers on DC and B cells from NP the ability to *trans* infect T cells. To provide further evidence that the reduced ability of APC from NP to *trans* infect T cells was related to cell cholesterol content, reconstitution experiments were done where DC and B cells from NP or SN were left untreated or incubated in cholesterol solution and extensively washed prior to loading with HIV-1 and coculturing with autologous CD4$^+$ T cells. By 8 days of coculture, reconstitution of cholesterol in DC and B cells from NP conferred on them the ability to *trans* infect T cells with HIV-1, e.g., 11,681 ± 6,413 and 9,047 ± 3,725 pg of p24, respectively, in T cells cultured with cholesterol-treated B cells compared to undetectable levels in T cells cultured with untreated B cells (Fig. 6A). When both APC and CD4$^+$ T cells from DC were treated with cholesterol, no significant difference from cultures where only the APC were treated with cholesterol was observed. Similarly, cholesterol reconstitution in CD4$^+$ T cells alone did not increase the efficiency of HIV-1 *trans* infection (Fig. 6A). As expected, addition of cholesterol to APC from SN did not alter *trans* infection (Fig. 6B). To confirm the efficacy of the cholesterol repletion in our cultures, an aliquot of the treated or untreated cells from both NP and SN was collected and the total cell cholesterol content was determined. The amount of cholesterol in treated DC and B cells from NP (Fig. 6C) was significantly higher than that in untreated cells, while it was minimally increased in treated CD4$^+$ T cells. In contrast, treatment of cells from SN did not result in a significant increase in cholesterol levels (Fig. 6D), supporting the concept that the increase in the efficiency of *trans* infection observed in the NP group was due to increased cholesterol levels.

To investigate further the basis of the reduced levels of cholesterol in APC from NP, we next determined the levels of ABCA1 mRNA by real-time reverse transcription (RT)-PCR. As shown in
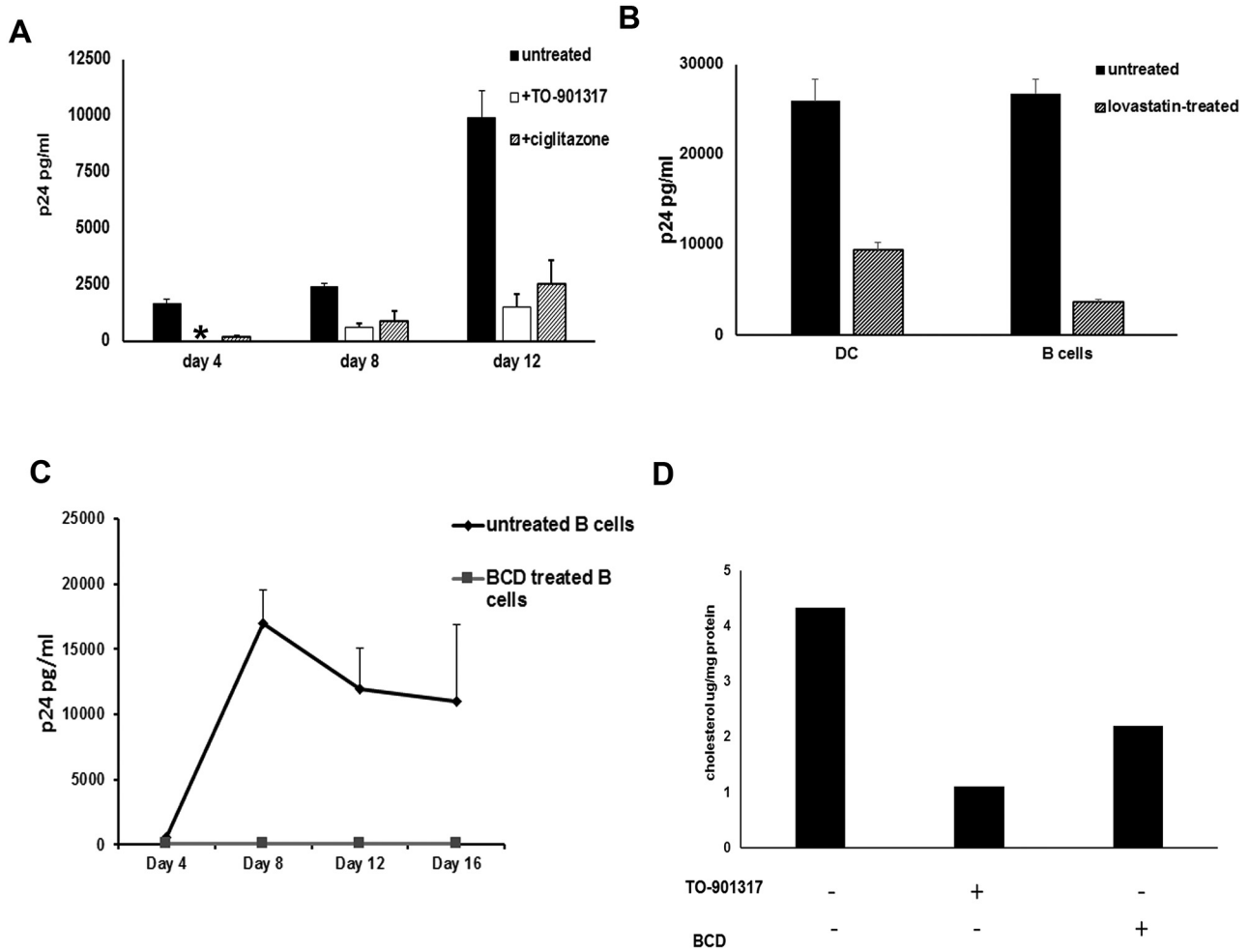
FIG 4 Alteration of cholesterol levels inhibits B-cell-mediated *trans* infection of T cells. (A) NR ligand treatment inhibits *trans* infection. B cells from three SN were either left untreated or treated with the LXR ligand TO-901317 or the PPARγ ligand ciglitazone prior to pulsing with HIV-1. Supernatants were collected at the indicated time points for assessment of p24 concentrations. Each result is the average of three sets of triplicate wells per time point (one set per subject). (B) Statin inhibits *trans* infection. B cells and DC from two SN were left untreated or treated with lovastatin prior to pulsing with HIV-1. Treated cells and controls were then cocultured with autologous CD4+ T cells in RPMI supplemented with 10% charcoal-stripped FBS. Supernatants were collected at the indicated time points for measurement of p24 concentrations. Each value is the average of triplicate wells for two SN. (C) Cholesterol depletion inhibits *trans* infection. B cells from two SN were left untreated or treated with BCD, loaded with HIV-1, and cultured with autologous CD4+ T cells. Supernatants were collected at the indicated time points, and p24 concentrations were determined by ELISA. Each result is the average of two sets of triplicate wells from two independent experiments. (D) Cholesterol content in cells left untreated or treated with TO-901317 or BCD. Results of a representative experiment of two independent determinations are shown. *, below the limit of detection.

Fig. 7A, APC from NP displayed higher levels of ABCA1 mRNA than those from PR, while the mRNA levels in the CD4+ T cells from the two groups were similar. To further analyze the role of ABCA1 in HIV-1 *trans* infection, we knocked down ABCA1 activity from NP with ABCA1 small interfering RNA (siRNA) and tested these cells in an HIV-1 *trans* infection assay. We found that knockdown of ABCA1 RNA activity in B cells from two NP increased their ability to *trans* infect autologous CD4+ T cells, resulting in 1,433 and 1,301 pg/ml of HIV-1 p24, compared to 85 and 124 in B-cell–T-cell cocultures treated with siRNA specific for ABCA1 or scrambled siRNA, respectively (Fig. 7B). The efficiency of ABCA1 knockdown was confirmed by lower levels of ABCA1 expression in treated cells assessed by flow cytometry (Fig. 7C) and by parallel transfection measuring pPGFP plasmid expression (Fig. 7D).

Taken together, these data suggest that altered cholesterol metabolism accounts for lower levels of cholesterol in APC of NP and the greatly reduced ability of these APC to *trans* infect CD4+ T cells. Importantly, the results indicate that the depletion of cholesterol in NP was due to an increase in ABCA1 activity.

DISCUSSION

Our results reveal that APC-mediated HIV-1 *trans* infection of CD4+ T cells is profoundly impaired in NP, who are HIV-1-infected individuals who have been able to control disease progression, i.e., maintaining a stable number of CD4+ T cells in their blood in the presence of persistent HIV-1 viremia, for many years in the absence of ART. In contrast, we noted that the ability of APC from PR who underwent a range of HIV-1 disease progression, with a continuous decrease in CD4+ T cells and an HIV-1 load
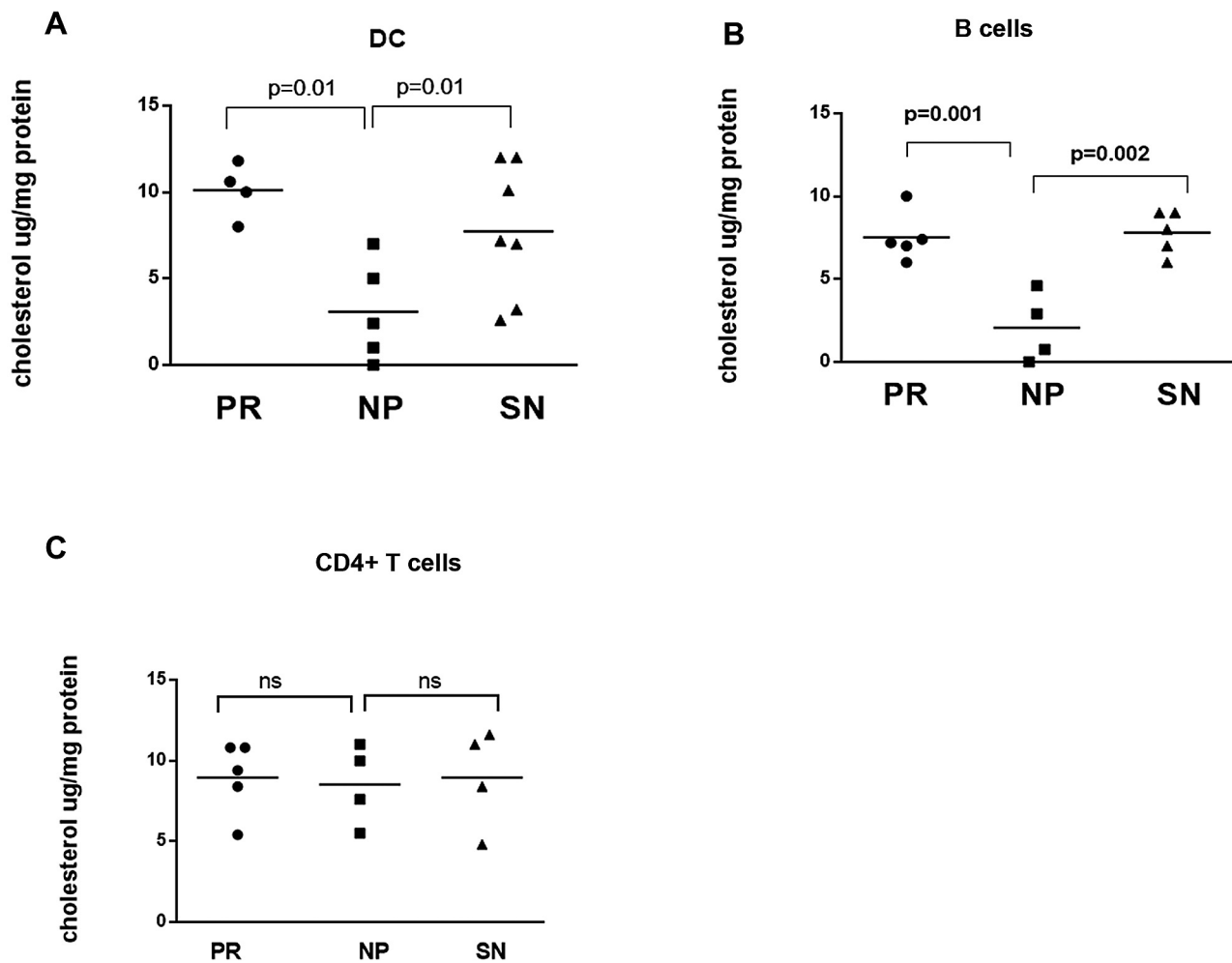
FIG 5 DC and B cells from NP have low cholesterol content. DC (A), B cells (B), and CD4$^+$ T cells (C) obtained from PR (PR1, PR2, PR3, PR7, and PR8), NP (NP1, NP2, NP3, NP6, and NP8), or SN were lysed with 1% Triton X-100; total cholesterol was measured with the Amplex Red assay kit; and results were normalized for total protein content. Differences between groups were compared by using a two-tailed *t* test. ns, no statistically significant difference.

increase, to *trans* infect T cells was comparable to that of APC from SN. This observation was not due to an intrinsic resistance of T cells from NP to HIV-1 infection, as demonstrated by comparable levels of virus production in T cells of NP, PR, and SN directly exposed to the same infectious concentrations of HIV-1. This concurs with other reports of similar levels of productive HIV-1 *cis* infection in CD4$^+$ T cells from NP and SN (19, 20). Cells from NP also failed to *trans* infect heterologous T cells from SN, further supporting the hypothesis that the impairment is at the level of the APC used as the transmitter cell. We provide further evidence that the effects observed were strictly derived from *trans* infection by excluding possible effects of *cis* infection with residual, endogenous HIV-1 in the cocultures derived from NP or PR. Furthermore, none of the NP carried the protective HLA B*5701 genotype and only one NP was heterozygous for the protective CCR5Δ32 mutation. These study group parameters exclude the potential influence of these known protective genotypes on our observations.

Our biochemical and molecular data demonstrate that the inability of DC and B cells from NP to transfer HIV-1 was directly related to alteration of cholesterol metabolism. These data include

(i) demonstration that treatment of B cells from SN with the NR ligand TO-901317 or ciglitazone, which increases cholesterol efflux, significantly reduced their ability to *trans* infect T cells; (ii) treatment of APC from SN with cholesterol synthesis inhibitors, i.e., statins, strongly inhibited the ability of these cells to *trans* infect CD4$^+$ T cells; (iii) a decreased content of cell cholesterol was directly linked to inhibition of HIV-1 *trans* infection and higher levels of ABCA1 in APC of NP; (iv) repletion of cholesterol in B cells from NP endowed these APC with the capacity to *trans* infect T cells; and (v) enhanced *trans* infection was coincident with inhibition of cholesterol transporter ABCA1 activity in APC from NP.

One of our most intriguing findings is that DC and B cells, but not CD4$^+$ T cells, from NP had significantly lower cholesterol content than DC and B cells derived from PR or SN. We postulate that this smaller amount of cholesterol in APC of NP inhibits their ability to form virologic synapses that transfer HIV-1 from APC to T cells (21). Indeed, cholesterol is essential in maintaining cell membrane integrity and cellular signaling, adhesion, and permeability, which are involved in B cell ontogeny (22) and the formation of immunologic synapses between APC and T cells (23, 24).
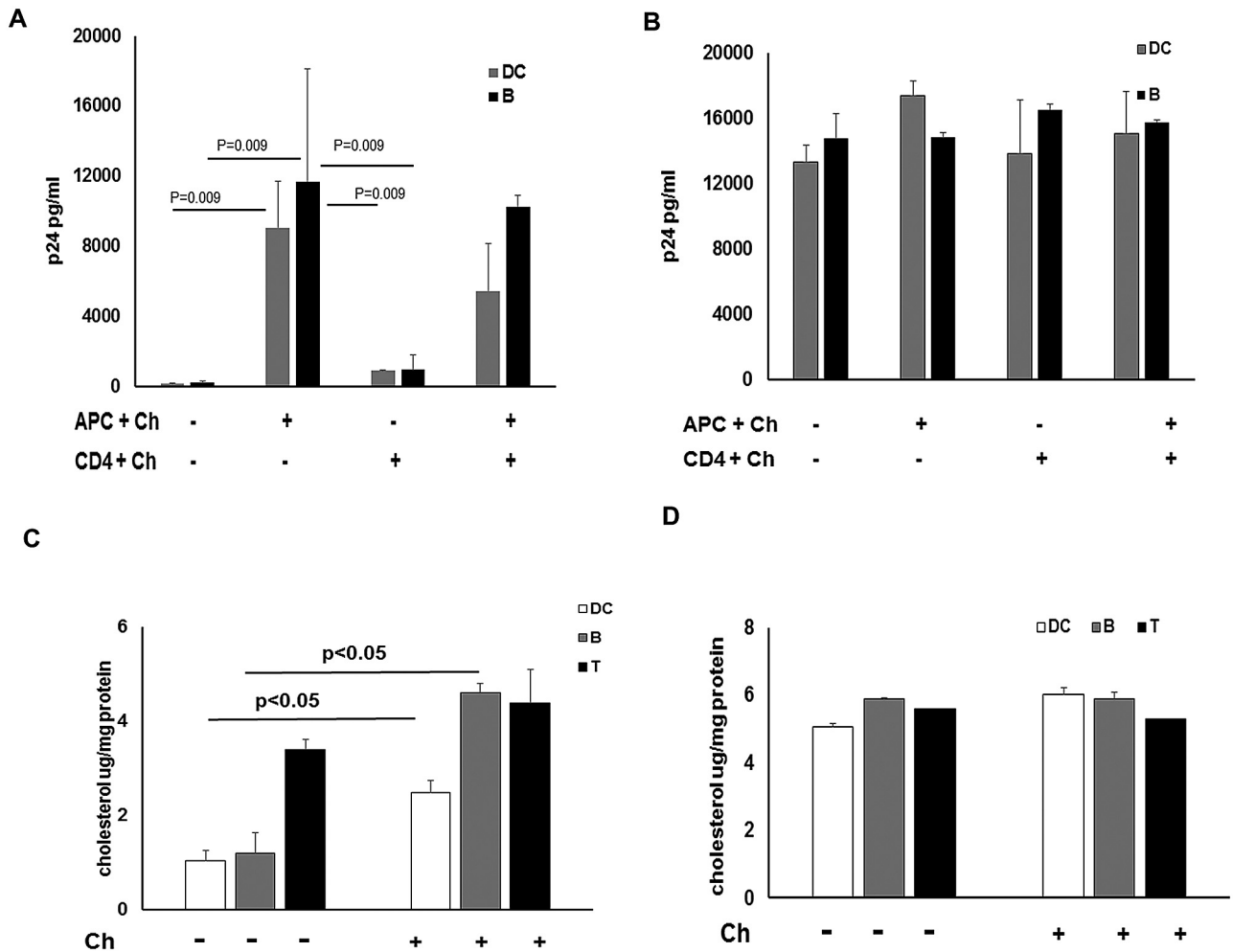
FIG 6 Cholesterol reconstitution in APC from NP confers on them the ability to *trans* infect T cells. (A) APC (DC and B cells) and CD4$^+$ T cells from four NP (NP1, NP2, NP3, and NP6) were left untreated or incubated in 300 μM cholesterol (Ch) for 1 h at 37°C. Cells were extensively washed, pulsed with HIV-1, and cocultured as shown. APC + Ch, APC treated with cholesterol; CD4 + Ch, CD4$^+$ T cells treated with cholesterol. (B) APC (DC and B cells) and CD4$^+$ T cells from three SN were left untreated or incubated in 300 μM cholesterol for 1 h at 37°C. Cells were extensively washed and cocultured as shown. APC + Ch, APC treated with cholesterol; CD4 + Ch, CD4$^+$ T cells treated with cholesterol. (C) Cholesterol contents of DC, B cells, and CD4$^+$ T cells from the four NP were measured before (−) and after (+) *in vitro* cholesterol reconstitution. *, $P < 0.05$. (D) Cholesterol contents of DC, B cells, and CD4T cells from two SN were measured before (−) and after (+) *in vitro* cholesterol reconstitution.

Moreover, APC regulation of cholesterol transport is mediated by environmental stimuli such as high-density lipoproteins, apolipoprotein A-1, and LXR signaling through endogenous ligand present in the microenvironment (25), while in T lymphocytes it is regulated during cell development (26). This difference in cholesterol metabolism between APC and T cells could account for the differences in cholesterol content that we observed in NP.

B cells obtained from PBMC of two NP that were cryopreserved both before and after HIV-1 seroconversion were not able to *trans* infect their contemporaneous, autologous CD4$^+$ T cells. However, B cells derived from two PR before and after seroconversion were equally capable of mediating HIV-1 *trans* infection. These data suggest that the inability of APC from NP to *trans* infect T cells with HIV-1 is an intrinsic, inherited trait related to lower levels of cholesterol in their APC and is not a result of HIV-1 infection. This implies that a small portion of the general population is inherently able to control HIV-1 infection for many years without ART on the basis of an inherited enhanced metabolism of

cholesterol. Work is ongoing to delineate host genetic markers that could be responsible for this inhibitory effect on HIV-1 *trans* infection. Recently, cortical actin and cytoskeleton remodeling have been shown to be important to the susceptibility of CD4$^+$ T cells to viral entry, in particular, in cell-to-cell transfer of virus (27–29). This could have a cholesterol component, and its involvement in the dynamics of HIV-1 *trans* infection in NP versus PR and SN would be interesting to investigate.

The ability of statins to reduce HIV-1 *trans* infection of CD4$^+$ T cells mediated by APC from PR and SN supports the concept that these drugs could be beneficial in the control of HIV-1 infection. There are conflicting reports of the effects of statins on HIV-1 *cis* replication *in vitro*, ranging from antiviral activity through a lymphocyte function-associated antigen (LFA) pathway (30) to no evidence of viral inhibition (31). Although statin treatment of HIV-1-infected persons has reduced viral loads and increased CD4$^+$ T cell counts (18, 32, 33), some clinical trials have not confirmed these observations (34). Statins also have pleiotro-
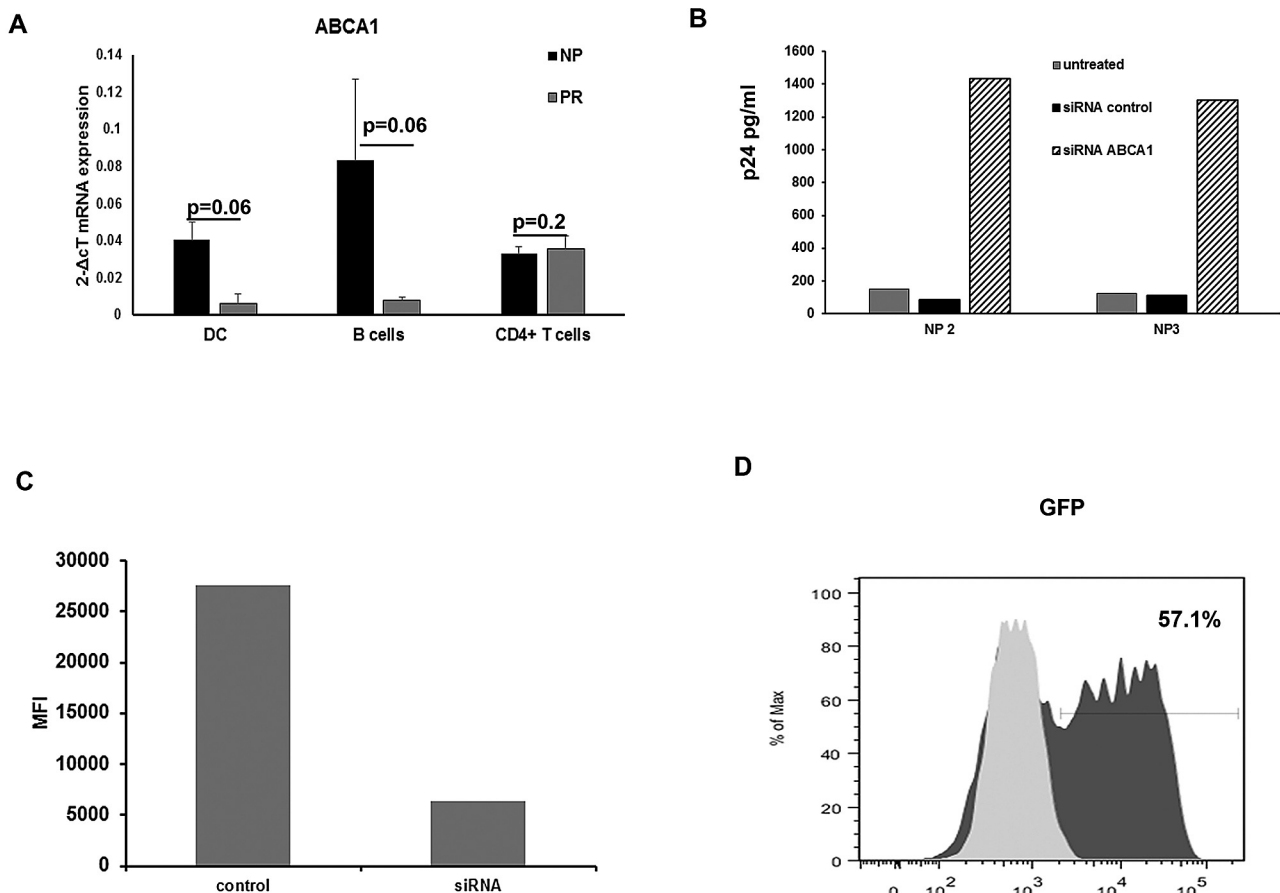
FIG 7 ABCA1 levels and effect of ABCA1 knockdown on HIV-1 *trans* infection. Levels of ABCA1 mRNA in three NP (NP2, NP3, and NP4) were determined by RT-PCR and compared to those in three PR (PR 2, PR3, and PR7). *P* values were determined by Student's *t* test. (B) ABCA1 knockdown restores *trans* infection. B cells from two NP (NP2 and NP3) were transfected with siRNA for ABCA1, treated with siRNA off-target controls, or left untreated; loaded with HIV-1; and cocultured with autologous CD4+ T cells for up to 12 days, when supernatants were collected and tested for HIV-1 p24 by ELISA. (C) Representative data from three independent experiments showing the mean fluorescence intensity (MFI) for ABCA1 on transfected B cells. (D) Efficiency of transfection was determined with parallel cultures transfected with the pmaxGFP plasmid (Lonza) and analyzed by flow cytometry. Positive cells (black histogram) were gated against the untreated control (gray histogram). GFP, green fluorescent protein.

pic effects that go beyond the lowering of cholesterol, such as modulation of CCR5 and RANTES expression and blocking of LFA-1 and ICAM-1 interaction (30, 35). Notably, none of the NP were receiving anticholesterol medications before or at the time of this study. It would be of interest to assess HIV-1 *trans* infection in persons taking statins.

In this report, we show a direct correlation between an inability of APC to mediate HIV-1 *trans* infection of T cells *in vitro* and stable CD4+ T cell numbers and low levels of HIV-1 viremia for many years in the absence of ART. We are not aware of other biological markers assessed to date that exhibit such an *in vitro* association with host control of HIV-1 infection. Furthermore, we show that this effect in NP is closely associated with lower levels of cholesterol in their DC and B cells and an increase in the ABCA1 levels in these subjects. Aptly, interference with cholesterol metabolism in SN *in vitro* rendered their DC and B cells unable to *trans* infect T cells with HIV-1, simulating what we found in NP. These data support previous observations that increasing ABCA1 activity by NR activation decreases HIV-1 infectivity *in vitro* (9–11). The present study supports these relationships among cholesterol levels, ABCA1 activity, and HIV-1 infectivity with the demonstra-

tion that altered lipid metabolism in professional APC, i.e., DC and B lymphocytes, results in a remarkable control of HIV-1 *trans* infection related to a lack of HIV-1 disease progression. This finding could be of importance in devising strategies for enhancing the control of HIV-1 infection.

MATERIALS AND METHODS

Study population. All subjects recruited from the MACS gave informed consent with approved University of Pittsburgh Institutional Review Board (IRB) protocols. Eight NP were recruited at the Pittsburgh site of the MACS (Pitt Men's Study) and assessed at approximately 6-month intervals over a 30-year period (Table 1). The duration of HIV-1 infection was defined in years of documented HIV-1 antibody seropositivity (positivity for HIV-1 antibody by enzyme immunoassay and confirmation by Western blotting) (13). The criteria for NP and PR are described above and in Table 1. Briefly, NP had a persistent CD4+ T cell count of at least 500 cells/mm³ with variable low numbers of HIV-1 RNA copies/mm³ of plasma (Roche) over 8 to 19.5 years of HIV-1 infection. PR had a persistent decline in CD4+ T cells, with high HIV-1 load levels over 2.1 to 8.5 years of infection. All of these HIV-1-infected subjects were ART naïve, and none were receiving treatment with cholesterol-lowering drugs.

Seven healthy blood donors (Central Blood Bank, Pittsburgh, PA) served as NC.

Preparation of DC, B cells, and T cells. In the MACS, blood was obtained at approximately 6-month intervals from NP and PR over >8 to 16.5 years (Table 1) to derive PBMC and plasma. Experiments in the present study were conducted from 2010 to 2014 with PBMC on the day of blood donation or with cryopreserved samples. Preliminary studies indicated that there was no difference in HIV-1 *cis* or *trans* infection between fresh and cryopreserved PBMC (data not shown). To derive DC, PBMC were first incubated with anti-CD14 monoclonal antibody (MAb)-coated magnetic microbeads (Miltenyi) according to the manufacturer's instructions. The resulting CD14+ monocyte fraction was used to generate DC as previously described (36). Immature DC were prepared from the CD14+ monocytes by culture for 5 days in the presence of 1,000 U/ml recombinant human IL-4 (rhIL-4; R&D Systems) and recombinant granulocyte macrophage–colony-stimulating factor in AIM-V medium (Gibco). B cells (CD19+ cells) were isolated from the CD14− cell fraction by incubation with anti-CD19 MAb-coated microbeads (Miltenyi). The purity of the fractionated B cells was >96.4% ± 0.4% as determined by staining with anti-CD20 MAb, with <1% CD14+ and CD3+ cells. Activated B cells were generated by culture of CD19+ cells in RPMI 1640 medium (Invitrogen) supplemented with 10% heat-inactivated fetal calf serum (FCS; GemCell), 1,000 U/ml rhIL-4, and 1 μg/ml soluble trimeric CD40 L (Axxora) for 48 h.

Autologous CD4+ T cells were obtained from the remaining CD14− CD19− fraction by CD4+ cell purification with anti-CD4 MAb-coated microbeads (Miltenyi). CD4+ T lymphocytes were cultured for 48 h in RPMI 1640 medium supplemented with 20% FCS, phytohemagglutinin (PHA; Sigma), and rIL-2 (Roche) as previously described (37) prior to use in HIV-1 *trans* infection experiments.

Flow cytometry. Expression of cell surface molecules was examined by flow cytometry with a Beckman-Dickinson SLRII. Cells were incubated with antibodies or isotype controls for 30 min at 4°C in phosphate-buffered saline (PBS) supplemented with 0.1% FCS and 0.1% NaN₃. After extensive washing with this buffer, cells were resuspended in 1% paraformaldehyde in PBS for flow cytometry and analyzed by FlowJo. The results were expressed as the percentage of positive cells above the isotype control threshold.

Virus. HIV BaL (R5-tropic virus) was propagated in PHA- and IL-2-activated normal donor PBMC and purified as previously described (37). Virus titers in pg/ml were determined by p24 ELISA (HIV-1 p24 Antigen Capture Immunoassay kits; SAIC-Frederick).

HIV-1 *trans* infection assay. DC or B lymphocytes (1×10^6) were incubated with HIV-1 at an MOI of 3 pg of p24/10^6 cells at 37°C for 2 h. This MOI does not result in productive *cis* infection of CD4+ T cells, as we have previously shown (6). HIV-1-loaded DC or B cells were washed extensively and incubated with activated, autologous CD4+ T cells at a 1:10 ratio. Cell-free supernatants were taken at various time intervals for titration of virus p24. No difference in viability between mock-treated and HIV-1-treated DC or B cells, as measured by trypan blue dye exclusion, was observed.

HIV-1 binding assay. DC or B cells (5×10^5 cells/well) were incubated with HIV-1 (7 ng p24) for 2 h at 4C. Cells were washed three to five times to remove unbound virus and lysed in buffer containing Triton X-100. Samples were analyzed for p24 content by ELISA.

NR receptor ligands. The NR LXR ligands TO-901317 and ciglitazone (Calbiochem) were reconstituted in dimethyl sulfoxide. DC and B cells were treated with 1 μM TO-901317 or 100 μM ciglitazone for 1 h prior to activation with CD40 L and IL-4 (9).

Cholesterol inhibition assay. DC and B cells were transferred to RPMI 1640 medium supplemented with 10% charcoal-stripped FCS (Invitrogen), treated with 10 μM lovastatin (Sigma, St. Louis, MO) for 24 h, washed, loaded with HIV-1, and used in *trans* infection experiments. Alternatively, cells were incubated in 10 mM BCD (Sigma) for 1 h at 37C,

washed extensively, and cocultured with autologous CD4+ T cells. Cell viability after treatment was >75%.

Cholesterol repletion assay. DC or B cells were incubated with 300 μM cholesterol in BCD for 1 h at 37°C as previously described (9), extensively washed in PBS, and then used in HIV-1 *trans* infection studies.

Cholesterol content determination. DC and B cells were washed 2× in PBS, and 1×10^6 cells were lysed in 200 μl of 1% Triton 100 in PBS. Fifty-microliter volumes of cell lysates were used for determination of total cholesterol content with the AmplexRed cholesterol assay kit (Invitrogen) in accordance with the manufacturer's instructions. Cholesterol content was normalized to total protein content, as determined with the Micro BCA protein assay kit (Pierce) according to the manufacturer's instructions.

siRNA knockdown of ABCA1. B cells were transfected with a pool of three to five target-specific, 19- to 25-nucleotide siRNAs directed against ABCA1 or a mixture of off-target siRNA controls (Santa Cruz Biotechnology). Purified B cells were cultured for 24 h in activation medium and then transfected with a 4D Nucleofector with the P3 4D-Nucleofector X solution kit (Lonza) according to manufacturer instructions. After transfection, cells were cultured in the original activation medium for another 24 h prior to use in HIV-1 *trans* infection experiments. Cells were also stained with anti-ABCA1 polyclonal antibody (Abcam) to determine the efficiency of ABCA1 knockdown and analyzed by flow cytometry.

cDNA synthesis and real-time RT-PCR. Real-time RT-PCR was performed on total RNA samples with commercially available TaqMan assays for ABCA1 (Applied Biosystems) on an ABI Prism 7000 sequence detection system (Applied Biosystems) as previously described (38). The level of ABCA1 expression was measured relative to the expression of the endogenous control gene for β-glucuronidase (β-GUS). The data were analyzed relative to β-GUS in the form $2^{-\Delta CT}$. Real-time RT-PCR were performed once with ABCA1, and β-GUS activity was assayed on the same plate for each sample, each in duplicate and with concurrent controls that lacked reverse transcriptase in the cDNA synthesis reaction mixture.

Statistics. Statistical analyses were performed with the two-tailed Student *t* test, two-way ANOVA, and linear regression. *P* values were calculated with GraphPad Prism software. *P* values of ≤0.05 were deemed statistically significant.

Study approval. This study was approved by the University of Pittsburgh IRB.

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