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Retention of Functional DC-NK Cross-Talk Following up to 18 Weeks Therapy Interruptions in Chronically Suppressed HIV Type 1$^+$ Subjects

EDITOR: Chronic viral replication during HIV-I infection results, among other effects, in the depletion of dendritic cell (DC) subsets,[1, 2] and decrease of natural killer cells (NK) frequency and function.[3] An inverse correlation between chronic viral replication and numbers of mature NK or DC cells[1,3] together with a lack of DC-associated functions has been reported.[1,4] However, introduction of suppressive antiretroviral therapy (ART) is associated with a variable degree of recovery of frequency and function among innate effector cells.[1] Following ART initiation, delayed kinetics of recovery of the innate compartment are observed in contrast to acute changes on T cell activation, suggesting a differential relationship between ART-mediated control of viremia and recovery of innate effector function.[5] Although the effect of structured therapy interruptions (TI) in ART-suppressed subjects has been described for adaptive immune responses[6] indicating an acute onset of T cell activation, no data are available regarding the effects of acute viral replication on innate immune system parameters following the absence of therapy. We therefore analyzed the effects of temporal viral replication on levels of innate cell immune reconstitution in ART-treated HIV-infected subjects who were participating on an observational study of the immune consequences of repeated therapy interruptions.

More precisely, we assessed changes in innate cell numbers and function during TIs in fresh blood samples from an observational cohort of 14 chronically HIV-1-infected patients (age $\geq$18 years) on suppressive ART (plasma HIV-1 RNA $<$50 copies/ml). ART initiation for all patients ranged from 1986 to 2000. All patients underwent two consecutive TIs of 6 and 18 weeks, with intervening ART resumption and resuppression to $<$50 copies HIV mRNA/ml for 4 weeks. All parameters were assessed at the start of each TI as well as at weeks 4 and 6 of the first TI, and weeks 4, 6 and 18 of the second TI. Informed consent was obtained according to the Human Experimentation Guidelines of the U.S. Department of Health and Human Services and of the authors' Institutions. The study protocol was approved by the Institutional Review Boards of the Wistar Institute and Philadelphia FIGHT.

Immunophenotyping characterization of NK, DC [plasmacytoid (PDC) and myeloid (MDC)], and T cell subsets was performed by same day whole blood four-color staining as previously described[7] by using the following combinations of directly fluorochrome-conjugated antihuman cell surface monoclonal antibodies from Becton Dickinson (BD) Biosciences (San Diego, CA): (1) CD56-fluorescein isothiocyanate (FITC)/CD16-phycoerythrin (PE)/CD3-PerCP/CD161-allophycocyanin (APC) (NK cells), (2) CD56-FITC/CD161-PE/CD3-PerCP/HLADR-APC (activated NK cells), (3) Lin-1 (CD3/CD14/CD16/CD19/CD20/CD56)-FITC/CD123-PE/HLA-DR-PerCP/HLA-ABC-APC (PDC), (4) Lin-1-FITC/CD11c-PE/HLA-DR-PerCP/HLA-ABC-APC (MDC), and (5) CD28-FITC/CD4-PE/CD3-PerCP/CD38-APC (T cells). Definitions of total NK cells included all CD3$^-$/CD161$^{+/-}$/CD56$^{+/-}$/CD16$^{+/-}$ NK subsets (with the exception of CD3$^-$/CD161$^-$/CD56$^-$/CD16$^{+/-}$ NK subsets, which were not included) whereas definitions of total HLA-DR$^+$ NK cells included all CD3$^-$/HLA-DR$^+$/CD56$^{+/-}$/CD161$^{+/-}$subsets (with the exception of CD3$^-$/HLA-DR$^+$/CD56$^-$/CD161$^-$, which were not included)]. PDC and MDC were defined as Lin-1$^-$/HLA-DR$^+$/CD123$^+$ and Lin-1$^-$/HLA-DR$^+$/CD11c$^+$, respectively, whereas CD8 cells were defined by gating on CD3$^+$/CD4$^-$ events. Results were expressed as mean fluorescent intensity (MFI), percent positive (%), and cells/mm^3. In the text cell numbers represent cells/mm^3.

PDC function within fresh peripheral blood mononuclear cell (PBMC) preparations was assessed by measurement of spontaneous (no stimulation), CpG oligodeoxynucleotide class A (CpG-2216, 10 μg/ml, Integrated DNA Technologies, Coralville, IA), or heat-inactivated PR8 influenza virus (10 HA units/ml)-induced IFN-α levels following 18 h of culture. Cell-free supernatants were harvested and tested in duplicate for IFN-α using commercial ELISA plates (PBL Biomedical Laboratories, Piscataway, NJ). Sensitivity of the assay was approximately 9.7 pg/ml.

NK function was assessed measuring spontaneous (no stimulation) and CpG-2216 (10 μg/ml)-induced NK cell-mediated cytotoxicity by standard ^{51}Cr release assay, as previously described, using fresh PBMC preparations as effectors cells against the tumor derived erythroblastoid cell line K562.[8] Results were expressed as area under the curve (AUC) for effector:target ratios of 50:1, 25:1, 12:1, and 6:1 for both spontaneous and induced NK function.

All statistical tests were performed using GraphPad Prism4 software. Briefly, a one-way ANOVA (Kruskal–Wallis test) was performed followed by Dunn's multiple comparison test. Differences were considered significant if $p < 0.05$. Short-term viremia following TIs did not significantly ($p > 0.05$) affect the number of CD4$^+$ T cells, although as expected clear changes in the activation state of both CD4$^+$ and CD8$^+$ T cells were noted as indicated by cell surface CD38 expression (Fig. 1A) and numbers of CD38$^+$ T cells. In contrast, short-term viremia had no effect on DC (Fig. 1B), NK, or activated HLA-DR$^+$ NK cells (Fig. 1C), and retention of NK cytotoxicity (Fig. 1D) and flu-stimulated IFN-α production (Fig. 1E) was observed

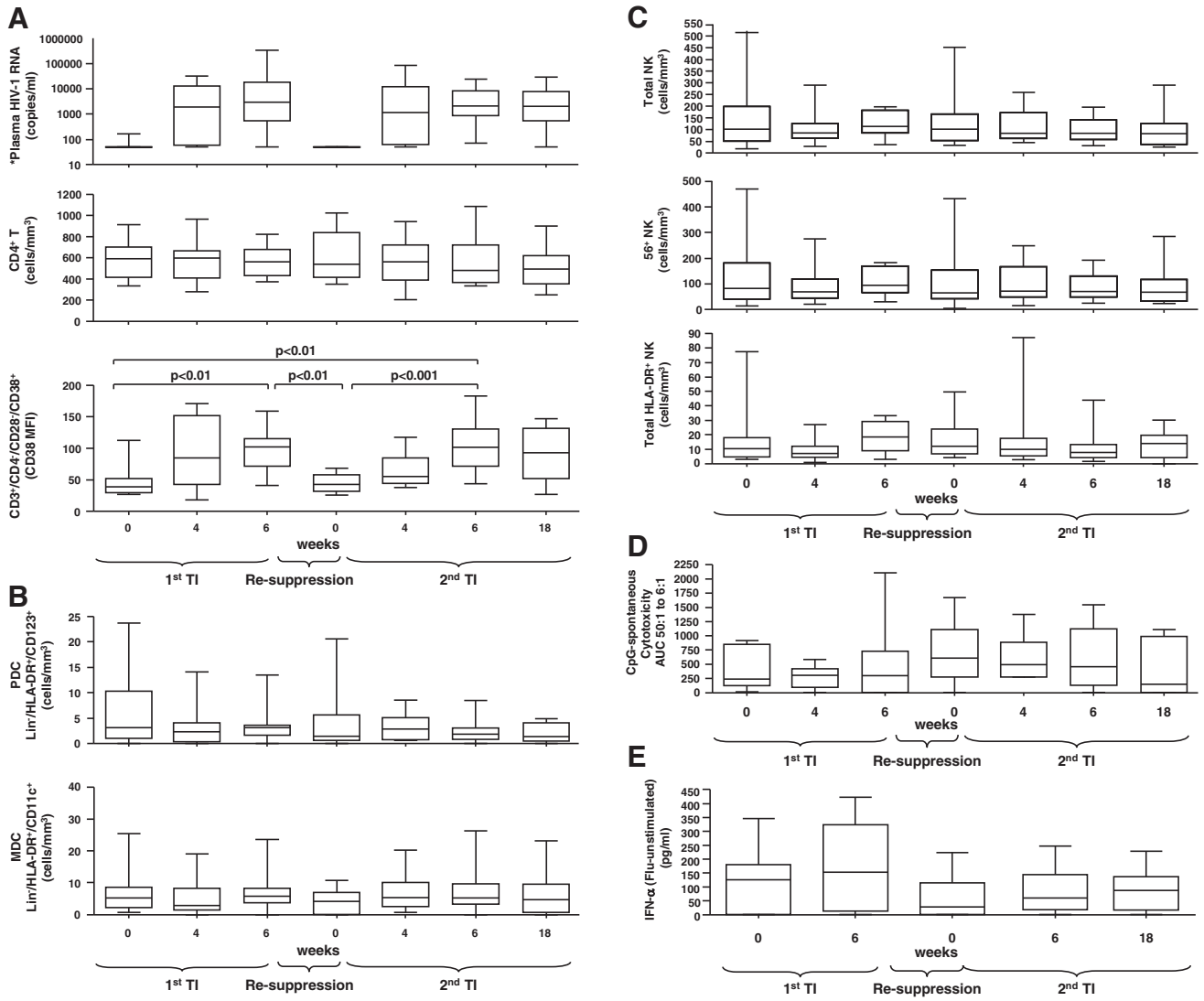

FIG. 1. Retention of innate effectors cell numbers and function during subsequent therapy interruptions and in spite of acute viral replication. (**A**) Plasma HIV-1 RNA (copies/ml), $CD4^+$ T cells (cells/ mm^3), CD38 mean fluorescent intensity on $CD3^+/CD4^-/CD28^-/CD38^+$ T cells; (**B**) PDC (defined as $Lin^-/HLA\text{-}DR^+/CD123^+$ cells/mm^3), MDC (defined as $Lin^-/HLA\text{-}DR^+/CD11c^+$ cells/mm^3); (**C**) total NK cells, total 56^+ NK cells, total HLA-DR^+ NK cells; (**D**) CpG-2216-induced NK function expressed as area under the curve (AUC) for effector:target ratios of 50:1, 25:1, 12:1, and 6:1 [(CpG-2216-induced cytotoxicity) – (spontaneous cytotoxicity)]; and (**E**) flu-induced PDC function expressed as IFN-α levels (pg/ml) [(flu-induced) – (unstimulated)]. Data are shown in (**A–D)** as interquartile (IQR) boxes (median, and outliers) at baseline, week 4, and week 6 of the first therapy interruption (TI) and at baseline, week 4, week 6, and week 18 of the second TI in 14 HIV-1-infected chronically suppressed patients. Data are shown in (**E)** as IQR boxes at baseline and week 6 of the first TI and at baseline, week 4, week 6, and week 18 of the second TI in 14 HIV-1-infected chronically suppressed patients. *Although plasma HIV-1 RNA increased significantly during TIs, this is not illustrated in the figure for simplification.

irrespective of viral rebound during TI periods or following suppression. Finally, no association between DC or NK cell numbers or function at baseline of each TI and resulting viremia was observed.

Overall, our data show that acute viral replication during TIs of up to 18 weeks is not associated with acute changes in the number of innate effector cell subsets or the onset of immediate impairments of NK and DC cell-mediated responses. Our data support our and other groups' observations showing lack of an immediate immunological "cost" of short-term TIs, as exemplified by retention of recall responses[9–14] and control of LPS levels during short-term TIs.[15] As a result, short-term viremia is unlikely to acutely deplete the innate effector cell subsets at the same rate it affects changes in distribution and activation within the T cell compartment. Although delays in the levels of increase of innate cell immune reconstitution as compared to uninfected subjects have been described after ART initiation and may vary between ART-treated subjects,[1,2,5] our data suggest that short-term viremia is unlikely to result in a sustained innate change. Therefore, a period longer than 18 weeks of viremia is likely required to significantly erode realized gains from previous innate cell immune reconstitution levels. Importantly, our data support the hypothesis that innate function can con-

tribute to immune control mechanisms (i.e., opportunistic infections) in spite of short-term viremia, while also serving as a potential target for immunotherapy, which may boost innate mechanisms of viral control. As a result, therapeutic trials targeting an activation of antiviral innate and/or adaptive responses (e.g., five such trials are currently recruiting as listed in clinicatrials.gov) on therapy interruption would not be expected to be acutely countered by an impairment in innate function.

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Author Disclosure Statement

No competing financial interests exist.

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